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THE PART PLAYED BY CAROTID BODY REFLEXES IN THE RESPIRATORY RESPONSE OF THE DOG TO ANOXEMIA WITH AND WITHOUT SIMULTANEOUS HYPERCAPNIA[1]

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Although Heymans' original conclusion (16) that the hyperpnea of anoxemia is essentially a chemoreceptor reflex phenomenon has now been accepted almost universally, several important points in this connection are still unsettled. One of these is the direct action of anoxia on the respiratory center—whether this is purely depressant, as the preponderance of evidence seemed to us to indicate (19), or whether direct stimulation of the center plays a part in the defense of the organism against anoxemia, as Dautrebande (5) and Gesell (13) have claimed. Another is the threshold of sensitivity of the chemoreceptors to anoxemia—whether this is so low that a considerable amount of chemoreceptor activity is maintained even by the arterial oxygen tension normally present during eupnea at sea level, as Euler *et al.* (8) (9) (10) and Gesell and co-workers (14) believe, or whether the threshold is relatively high, as indicated by the absence of respiratory depression in normal men when they breathe oxygen at sea level (15) (21) and by the lack of hyperpnea in aviators until they reach an altitude higher than 4000 feet (1). Another point that deserves further study is the combined effect of anoxemia and hypercapnia simultaneously elicited —a combination which Dill and Zamcheck (7) have recently found to be increasingly stimulant in man in direct proportion to the intensity of either stimulus, at least until the anoxemia became very severe. The addition of CO_2 to mixtures low in O_2 definitely improved arterial O_2 saturation in

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their subjects, and Bergeret (3) reports similar findings in aviators. We hoped to elucidate this relationship by securing data on the extent to which it depends on chemoreceptor reflexes—a point which cannot be decided by existing evidence, for Gellhorn and Lambert (11) state that the effects of anoxemia and hypercapnia are still additive (in dogs) after chemoreceptor denervation while Smyth (22) found that anoxia only depressed the respiratory response (of rabbits) to CO_2 after the denervation.

Discrepancies such as these are the result of drawing conclusions of general importance from a few illustrative examples, a procedure that has been characteristic of most of the literature on chemoreceptor reflexes in the past. For reasons that have been presented elsewhere (19), we are convinced that the rôle of these reflexes can be determined only by experimental evidence that is adequately quantitative in amount as well as nature, and it was our purpose in these experiments to obtain such evidence bearing on the points just mentioned. As in an earlier study (20) dealing with the effects of hypercapnia, we employed methods as simple and unobjectionable as we could make them.

Methods. These were similar in the main to those employed in our previous work (20) as far as animals (unselected dogs weighing 10 to 18 kgm.), recording of respiration (by pneumograph and measurement of air expired through a valved tracheal cannula) and blood pressure (by a Hg manometer from a femoral artery). are concerned. In the present study however we used smaller doses of chloralose (20 25 mgm. per kilo injected intravenously during light ether anesthesia following a preliminary injection of 2 mgm. of morphine per kilo subcutaneously). These animals were therefore under very light anesthesia and the experiment had to be completed within 4 hours to obviate the necessity for more chloralose. Another change in technic was the use in these experiments of the ligation-collapse method of Gesell, Lapides and Levin (14) for inactivating the carotid pressoreceptors instead of the more laborious and dangerous method of section of nerve fibers used in our earlier experiments. We also succeeded in some experiments (nos. 1, 2, 4, 5, 6, 8 and 14) in separating one depressor nerve from the vagus trunk; in these cases the former nerve was cut and the latter was spared, but the other vagodepressor was always cut. Inf the others (nos. 3, 7, 9, 10, 11, 12 and 13) both vagodepressor nerves were cut. The adequacy of chemoreceptor denervation was tested by intravenous injection of NaCN in dosage (4 6 mgm. total) sufficient to produce strong hyperpnea before denervation; in only one experiment (no. 10) was there any appreciable stimulant effect from this after the carotids were denervated, and in this case the one vagus, previously left intact after cutting a group of fibers which responded like the depressor to electrical stimulation, was then cut before the experiment was carried further, following which the stimulant effect of NaCN was entirely gone. This is the only case in whichgthe condition of the vagi was altered during the course of the experiment. Carotid denervation was accomplished by complete division, between ligatures, of all attachments of the carotid reflex zone, vascular as well as nervous; since the method used to inactivate the carotid sinus pressoreceptors involved ligation of the common, internal, and external carotid arteries, the cerebral circulation was not further modified by this method of denervation. The reactivity of each animal was tested by intravenous injection of NaCN (chemoreceptors) and by inhalation of

CO_2 in O_2 (center); experiments in which low reactivity was indicated by either test were discarded.

Blood samples were collected from a large cannula in a femoral artery into pyrex tubes under oil, heparin (0.1 cc., 100 units) being added to each to prevent clotting, and on each occasion two samples of about 10 cc. each were collected. One of these was used for estimation of oxygen saturation, the other (in which melted paraffin wax was immediately poured into the oil over the blood) for estimation of the CO_2 content and pH of the plasma separated by centrifugation; the analyses were begun as soon as the samples were collected and the specimens were kept in the ice box until they were analyzed. Blood gas analyses were made by the manometric method of Van Slyke (18), pH determinations in a closed glass electrode at 38°C.; the accuracy of each pH reading was checked against estimations on two buffer solutions of known pH. Arterial O_2 saturation was estimated in the usual way (18) by comparing the O_2 content of the blood with that of the same blood after saturation in room air at room temperature; this was routinely determined on the first and last samples in each experiment and since no appreciable changes were found similar estimations were not made on the intervening specimens. To change the gas content of the blood we permitted the animal to inhale, via the inlet valve and from a Douglas bag, various gas mixtures, as follows: Room air, pure (tank) O_2, O_2 + 3.5 per cent CO_2 (about), mixtures low in O_2 (10, 12, or 14 per cent in N_2), and the same mixtures to which we added about 3.5 per cent CO_2. The gas content of the CO_2-containing mixtures was determined in each experiment by means of a Henderson-Haldane gas analyzer (18); each of the O_2–N_2 mixtures was prepared in a large cylinder at the factory and the same mixtures were used throughout these experiments.

The course of a typical experiment was as follows: After we had tested the animal's reactivity to NaCN and had permitted a period of quiet breathing of air for at least 10 minutes, the first blood samples were taken. The blood was replaced by transfusion (via a femoral vein) of 20 cc. of fresh heparinized dog's blood, and this was also done after each subsequent collection. Then the animal was allowed to breathe pure O_2 from a Douglas bag and a second sample was collected at the end of 5 minutes; breathing usually became steady within 2 minutes but here, as in the other experimental periods, we tried to be certain that our data pertained to a steady state. Then (without a pause) CO_2 in O_2 was substituted for the O_2 and the third blood sample was collected at the end of 5 minutes (occasionally 6 if breathing had not been steady for 2 min. at this time). After a pause of 10 minutes the animal was made to breathe a mixture low in O_2 but without added CO_2; a fourth blood sample was collected after 5 minutes of this. Then (without a pause) a similar mixture containing CO_2 was applied and a fifth blood sample was collected after 5 minutes. This completed the series of observations with the chemoreceptors functioning. The latter were now denervated and the completeness of the process was tested by intravenous injection of NaCN. Then the entire series of inhalations and blood collections was repeated; the CO_2–O_2 mixture used after the denervation was in all cases the same as that used before, but after several animals rapidly succumbed after denervation to inhalation of an O_2–N_2 mixture that had previously been well tolerated, we routinely used gas mixtures containing 2 per cent more O_2 after the denervation than before it, both with and without added CO_2. Thus 10 per cent O_2 before denervation was followed by 12 per cent afterward, or 12 per cent by 14. Arterial pCO_2 was estimated from the plasma CO_2 content and pH, by the Henderson-Hasselbalch formula (18). Arterial pO_2 was calculated from the O_2 saturation and the pH, using the data and formula given by Dill *et al.* (6). Measurements of respiration (rate and minute volume) were made on the kymographic

tracing for 2-minute periods except for a few instances in which (with low O_2 after chemoreceptor denervation) it was necessary to terminate the observations while breathing was showing progressive depression; in such cases a one minute period had to suffice.

Thus we were enabled to secure data on: *1*, the behavior of respiration (rate and minute volume) and blood pressure when a lightly anesthetized animal whose chemoreceptors were known to be capable of strong activity was made to breathe successively room air, pure O_2, 3.5 per cent CO_2 in O_2, 10 or 12 per cent O_2 in N_2, and the same plus 3.5 per cent CO_2; *2*, the part played by chemoreceptor reflexes in these phenomena; *3*, the corresponding changes in arterial O_2 saturation, pO_2, pH, and pCO_2. Fourteen technically satisfactory experiments were made; in 3 of them the mixtures used to elicit anoxemia contained 12 per cent O_2 (before denervation) and 14 per cent (after denervation), while in the remaining 11 the corresponding percentages were 10 and 12; this change was made because the former mixtures turned out to be poorly effective or ineffective in producing anoxemia in our animals. These particular experiments are chosen for this report only because they were free of objection on technical grounds; the data to be presented below are unselected otherwise. A considerable number of other experiments had to be discarded for various reasons. In some there was trouble with narcosis, which was either so deep that the animal's breathing did not respond to 3.5 per cent CO_2, or so light that breathing was too irregular to enable us to obtain valid data. In others the circulation was greatly depressed, either from the start or following the anoxic periods. In still others the analytical data were incomplete or otherwise faulty. In a few the chemoreceptors must have been damaged during the dissection for intravenous injection of NaCN elicited little or no hyperpnea.

RESULTS. *1. The effects of alterations in the arterial oxygen tension (pO_2).* a. *Increased pO_2.* When 100 per cent O_2 was substituted for room air at the start of the experiment (i.e., with the chemoreceptors intact) respiratory minute volume was decreased in 8 of the animals and increased in 6 (tables 1 and 2). The changes exceeded 10 per cent in only 2, however, and were almost equal in opposite directions in these. Changes of 10 per cent are little if at all beyond the range of error of these measurements of the volume of expired air and we do not regard them (or smaller changes) as significant. In a number of these animals there was a considerable depression of breathing immediately after oxygen inhalation was begun but in every case but one this was transitory; the data given in table 2 portray the findings during the steady state reached within 5 minutes after the oxygen inhalation began. The temporary depression that sometimes ensued when oxygen was first inhaled indicated that some chemoreceptor activity had been present in these particular animals; the transitory nature of the effect indicates that the reflex factor was non-essential under the conditions of these experiments. The changes in the blood shown in tables 1 and 2 afford additional evidence against an important grade of chemoreceptor activity maintained by the arterial pO_2 associated with quiet breathing of air because arterial cH and pCO_2 showed no definite and consistent increase when oxygen was breathed. It is noteworthy that in every case in which arterial pO_2 was at or below 60 mm. Hg during the period of

TABLE 1

Respiration and arterial blood during quiet breathing of air and the effect of chemoreceptor denervation on them

In this table, as in all others in this paper, the first 3 experiments are treated separately because the grade of anoxemia was less severe in them than in the others (see tables 3 and 5); the experiments were all identical in all other respects. The numerical designation of each experiment is the same in all the tables. In every case the figures for respiration are the findings during the steady state existing during the 2 minutes just before the blood sample was collected, which was usually 5 or 6 minutes after the start of the inhalation of the gas mixture under investigation. The percentage changes in respiration indicated in the tables are based on minute volume and the control level from which they were calculated is indicated in each case; in table 1 the changes shown in the last vertical column are those observed during quiet breathing of room air 5 to 15 minutes after the denervation as compared with the corresponding figure just before denervation. The incompleteness of the analytical data in experiment 1 was due to a misunderstanding, that in experiments 11 and 13 to breakage during centrifugation of the tube containing the blood on which arterial CO_2 content and pH were to be determined.

EXPERIMENT	ARTERIAL BLOOD								RESPIRATION				
	Sat. with O_2, per cent		pO_2, mm. Hg		pCO_2, mm. Hg		pH_a		Normal		Denervated		Change
	Normal	Denervated	Normal	Denervated	Normal	Denervated	Normal	Denervated	Rate	Min. vol.	Rate	Min. vol.	
										cc.		*cc.*	*per cent*
1	95	94							9	3000	16	5000	+66
2	96	96	80	90	32	32	7.48	7.34	7	3000	10	4000	+33
3	94	96	74	84	42	38	7.33	7.36	10	2750	10	3250	+18
Average, 1-3.....	95	95	77	87	36	35	7.40	7.35	9	2920	12	4080	+39
4	90	89	50	51	29	31	7.49	7.47	10	3750	13	3250	−13
5	88	89	53	43	29	25	7.42	7.46	8	3000	8	3250	+8
6	96	97	90	90	41	38	7.36	7.37	5	2500	6	2750	+10
7	82	96	60	95	45	28	7.19	7.20	4	2200	6	2800	+27
8	82	96	62	90	45	31	7.25	7.32	5	3500	9	5500	+66
9	82	90	50	61	37	35	7.34	7.36	5	4500	6	5000	+11
10	91	87	66	60	44	36	7.32	7.30	29	4750	25	5000	+5
11		95		80		40		7.32	8	2650	9	3500	+32
12	94	94	85	85	57	50	7.20	7.20	7	3620	14	5750	+37
13	86	83	63		45		7.23		16	4000	17	4750	+19
14	91	90	76	76	40	36	7.27	7.24	9	4200	8	5000	+19
Average, 4-14....	88	91	66	73	41	35	7.31	7.32	9	3520	11	4230	+20

air breathing, respiration was considerably depressed at first by oxygen, and the single instance of a considerable and maintained depression (expt. 4) was in the animal that showed the lowest arterial pO_2 encountered

during the control period (50 mm. Hg). The data are consonant with the belief that a persistent depression of breathing by oxygen occurs only when arterial pO_2 was distinctly subnormal before oxygen was given.

After chemoreceptor denervation oxygen inhalation led to an increase

TABLE 2

The effect of inhalation of 100 per cent O_2

The indicated percentile changes in respiratory minute volume are from the level during quiet breathing of air (table 1).

EXPERIMENT	ARTERIAL BLOOD						RESPIRATION					
	Sat. with O_2,* per cent		pCO_2, mm. Hg		pH_a		Normal			Denervated		
	Normal	Denervated	Normal	Denervated	Normal	Denervated	Rate	Min. vol.	Change	Rate	Min. vol.	Change
								cc.	*per cent*		*cc.*	*per cent*
1	100	100	33	20	7.44	7.48	10	2900	−3	15	5100	+2
2	100	100	32	22	7.46	7.45	7	3250	+8	11	4750	+19
3	100	100	38	33	7.36	7.39	10	3000	+9	9	4000	+23
Average, 1–3.....	100	100	34	25	7.42	7.44	9	3050	+4	12	4620	+13
4	100	100	31	28	7.48	7.48	10	3000	−20	13	3500	+8
5	100	100	32	23	7.38	7.43	7	2750	−8	8	3750	+13
6	100	100	41	34	7.38	7.40	5	2250	−10	7	2750	0
7	100	100	47	25	7.20	7.27	4	2400	+9	7	3550	+27
8	100	100	44	27	7.26	7.35	5	3300	−6	9	5900	+8
9	100	100	42	34	7.31	7.35	6	4150	−8	6	5700	+14
10	100	100	38	35	7.38	7.31	30	4550	−4	21	4600	−8
11	100	100	48	39	7.32	7.31	7	2550	−4	10	4250	+21
12	100	100	53	46	7.21	7.22	9	4500	+24	14	7250	+26
13	100	100	35	37	7.33	7.21	17	3750	−6	17	5250	+11
14	100	100	42	34	7.26	7.24	8	3800	−10	9	5750	+15
Average, 4–14.....	100	100	41	33	7.32	7.32	10	3360	−5	11	4840	+12

* The O_2 content of most of these blood samples was greater than that corresponding with 100 per cent saturation, the excess being presumably due to O_2 in physical solution. We have been unable to find an acceptable method for calculating arterial pO_2 under such circumstances and have therefore omitted this column from the table.

in pulmonary ventilation in 12 of the 14 experiments; in 9 the change exceeded 10 per cent and may be regarded as significant (table 2). In one case breathing was unchanged and in one it was slightly depressed (8 per cent). Since there was no increase in arterial pCO_2 or cH to account for the observed respiratory stimulation it most probably was due to in-

creased excitability of the center, and this can best be attributed in turn to an acceleration of the vegetative oxidations within the central neurons. The possibility that the responsible factor was an increase in pCO_2 within these cells because of decreased capacity of the hyperoxygenated blood to transport CO_2 away from them (2) (12) is denied by the fact that arterial pCO_2 was lower during the inhalation of oxygen than in the control period in all of the animals whose breathing was increased more than 10 per cent by oxygen. It is of course possible that the pCO_2 within the center may have risen independently of the arterial blood during O_2 inhalation, but if equilibrium between arterial blood and nerve cells with respect to CO_2 is not present a much more serious discrepancy must be conceded with regard to less diffusible agents such as O_2 and H^+. The rate of diffusion of the latter agents through these membranes would then appear to be so slow as to preclude any significant correlation of events within the center with the chemical composition of the arterial blood. We agree with Dill and Zamcheck (7) that the burden of proof is properly on those who claim that equilibrium does not exist between the pCO_2 within the center and in the arterial blood under any ordinary physiological conditions.

These results indicate that oxygen inhalation has two opposing effects on respiration—a removal of any preëxisting chemoreceptor drive aroused by the arterial pO_2 associated with the breathing of room air, and an increase in the functional capacity of the central neurons as a result of the increased arterial pO_2. In normal man at sea level the latter apparently predominates since his breathing is stimulated when he breathes oxygen (21); it is noteworthy that in our two experiments in which arterial pO_2 was highest when the chemoreceptors were intact (nos. 6 and 12) breathing was distinctly increased by O_2 inhalation in one, slightly (and probably not significantly) depressed in the other. The possibility that stimulation of breathing by O_2 inhalation is due to reflexes aroused by irritation of the respiratory passages is negatived by the presence of the phenomenon in animals both of whose vagodepressor nerves were cut at the start (expts. 3, 7, 9, 10, 11, 12, and 13) and its absence in some of those in whom one vagus remained intact (expts. 1, 2, 4, 5, 6, 8, and 14). If this is a reflex the afferent pathway must be something other than the vagus, sympathetic, or depressor nerves, which seems very unlikely.

b. *Decreased pO_2.* Our data on this subject are summarized in table 3. The results obtained with simultaneous anoxemia and hypercapnia are not included since they are to be treated separately (table 5). Of the 3 animals given 12 per cent O_2 before denervation only one (no. 2) showed a definite increase in pulmonary ventilation; this was also the only one of these 3 in which arterial O_2 saturation was considerably reduced or in which arterial pO_2 fell below 55 mm. Hg. We have no explanation to offer for the diminution of pulmonary ventilation associated with the breathing of 12

per cent O_2 in experiment 3; it is the only instance of depression by low oxygen with chemoreceptors intact but no reason for this occurrence in this experiment was evident at the time or in the subsequent analytical data. In experiments 4 to 14 the anoxemia was without exception more severe

TABLE 3

The effect of simple anoxemia (inhalation of 12 per cent O_2 in N_2 in experiments 1 to 3 before denervation, 14 per cent O_2 in N_2 after denervation; in experiments 4 to 14 the O_2 percentages were 10 and 12 respectively)

The indicated changes in respiratory minute volume are measured from the period of air breathing immediately preceding this; the figures were not always the same as those shown in table 1.

EXPERIMENT	ARTERIAL BLOOD								RESPIRATION					
	Sat. with O_2, per cent		pO_2, mm. Hg		pCO_2, mm. Hg		pH_a		Normal			Denervated		
	Normal	Denervated	Normal	Denervated	Normal	Denervated	Normal	Denervated	Rate	Min. vol.	Change	Rate	Min. vol.	Change
										cc.	*per cent*		cc.	*per cent*
1	93	90	61	54	24	31	7.45	7.45	6	6000	+9	19	4000	−20
2	85	85	46	53	29	31	7.48	7.36	8	3500	+17	10	3500	−18
3	88	79	57	42	37	29	7.37	7.42	9	2250	−35	10	4750	0
Average, 1-3.....	89	85	55	50	30	30	7.43	7.41	8	3910	−2	13	4080	−13
4	82	38	36	21	22	35	7.55	7.44	17	6250	+37	12	2250	−36
5	80	48	40	25	21	23	7.49	7.44	11	5500	+47	9	3500	0
6	68	57	33	30	28	39	7.46	7.39	8	4500	+50	7	2000	−33
7	64	68	39	43	34	27	7.28	7.26	6	3400	+36	6	2400	−23
8	65	61	37	33	33	30	7.33	7.36	8	5500	+22	10	4750	−10
9	69	64	37	37	34	38	7.38	7.33	6	5250	+17	5	4000	−27
10	79	35	42	23	32	43	7.41	7.29	32	6100	+22	21	3200	−20
11	57	60	29	38	37	40	7.41	7.27	11	4750	+50	10	3000	−20
12	48	51	28	39	42	44	7.32	7.27	10	5000	0	14	4000	−20
13	62	54	35	33	31	38	7.34	7.28	11	5600	+27	17	3750	−21
14	74	68	41	42	27	34	7.37	7.29	12	6250	+32	8	3750	−21
Average, 4-14.....	68	55	36	33	31	36	7.40	7.33	12	5280	+30	11	3300	−21

and distinct hyperpnea occurred in all but one (expt. 12), in which breathing was unchanged although arterial pO_2 was lowered more than in any other animal with intact chemoreceptors. Breathing actually was stimulated earlier in the anoxic period in this case also but the stimulation was temporary and by the time the blood sample was taken it had disappeared entirely; blood pressure also fell at this time (table 6). It would seem

therefore that in this animal the anoxemia was so severe that the central depressant effects of anoxia overcame the stimulant effects of chemoreceptor reflexes. The changes in arterial pH and pCO_2 are concordant with the belief that the dominant factor was a new reflex drive to the respiratory center, for pH consistently rose and pCO_2 consistently fell. No correlation of the intensity of the hyperpnea with the degree of anoxemia is apparent. In view of the well-known variations in the intensity of the hyperpnea produced in normal men by inhalation of mixtures low in O_2 (15) or by ascent in aircraft (1) this is not surprising. There is also no evidence of a simple relation between the strength of the hyperpnea and the change in arterial pCO_2 or pH such as would be expected if the hyperpnea were limited by the extent to which either of the latter factors was altered. The stimulation of respiration affected both rate and depth; average depth was increased by inhalation of 10 per cent O_2 from 390 cc. (table 1) to 440 cc. (table 3).

The most significant feature in these results is the change produced by chemoreceptor denervation. With only 3 exceptions respiration was now depressed significantly during the anoxic period, the average depth in experiments 4 to 14 being decreased from 385 to 300 cc.; in 2 of the exceptions there was no change, while in the third the diminution (10 per cent) cannot be called significant. In no case was there any respiratory stimulation. The diminution in arterial pO_2 showed a tendency to be greater after the denervation than before, this being the case in 8 of the 14 experiments; in one, the two values were identical, and in 5 the pO_2 during the anoxic period was somewhat higher after the denervation. This is noteworthy because the gas mixtures used after the denervation always contained 2 vols. per cent more O_2 than those used before it. The changes in arterial pH and pCO_2 associated with anoxemia after the denervation were not consistent; the average figures indicate no significant change and this we believe to be an accurate portrayal of the actual situation. Reference to the corresponding values during the control period (table 1) shows that the depression of breathing now produced by anoxia cannot be attributed to a diminution in the chemical stimulus in the arterial blood.

These data indicate that the smallest degree of anoxemia by which the chemoreceptors of lightly anesthetized dogs are measurably stimulated corresponds with an arterial O_2 saturation of about 85 per cent and an arterial pO_2 of 50 to 55 mm. Hg. The effect of the denervation indicates that the hyperpnea of anoxemia is regularly and entirely due to chemoreceptor reflexes; the effect of anoxia directly on the respiratory center appears to be purely a depressant one. Our finding that the degree of anoxemia produced by inhalation of a given O_2–N_2 mixture is significantly greater after chemoreceptor denervation than before confirms that of Wright (23) in similar experiments on cats. From a number of experi-

ments in which inhalation of 10 per cent O_2 after the denervation caused quite rapid failure of respiration (and circulation) and a few in which 5 per cent O_2 elicited similar effects even more rapidly, we are certain that the results shown in table 3 would only have been exaggerated if more severe anoxemia had been produced.

2. The effects of uncomplicated hypercapnia. Since one of the major purposes of this investigation was to determine whether hypercapnia and anoxemia have a synergistic effect on the respiratory center it was necessary to determine the effect of each of these separately. The data with respect to CO_2 in O_2 are therefore presented in table 4. The control values are those found during inhalation of 100 per cent O_2 (table 2). These data also serve another purpose that was not contemplated when the experiments were performed: they show that chemoreceptor denervation does not appreciably alter the respiratory response of the lightly anesthetized dog to inhalation of a low (about 3.5 per cent) concentration of CO_2 in O_2. The actual level of pulmonary ventilation associated with such inhalation tended to be considerably greater after the denervation than before, but because the control value was also greater the percentage change in respiration during CO_2–O_2 inhalation was not very different under the two circumstances. Perhaps the best evidence that chemoreceptor reflexes played no measurable part in the response of these dogs to a low concentration of CO_2 is the consistently lower value for arterial pCO_2 during the inhalation after the denervation. This may have been related to the stimulant effect of the O_2 alone (table 2), to continuous irritation of afferent nerves by the ligatures used in the denervation, or to a coincidental lightening of the narcosis as the experiment progressed. In any case the conclusion derived from our earlier study (20), viz., that chemoreceptor reflexes play no demonstrable part in the response of the lightly anesthetized dog to minimum effective increases in arterial pCO_2 as far as rapidity of onset and effective increase in pCO_2 are concerned, can now be extended to apply to the intensity of the hyperpnea so produced. As pointed out elsewhere (19), evidence obtained in unanesthetized animals also indicates that chemoreceptor reflexes play no measurable part in the respiratory response to hypercapnia. In deeply anesthetized animals, in which the reactivity of the central neurons to CO_2 is greatly depressed, the situation undoubtedly is different (17) (19). It is probable that the belief of Euler (8) and Gesell (14) that chemoreceptor reflexes are vitally concerned in the response to CO_2 under all circumstances is due to their having used animals deeply narcotized with chloralose. In view of the experimental data submitted in this paper and our earlier one (20) we believe that the burden of proof now should be on those who hold that the chemoreceptors are not considerably less sensitive than the center to CO_2.

3. The effects of anoxemia and hypercapnia simultaneously elicited. The

data on this point are summarized in table 5. The O_2 content of the inspired air was in all cases the same as that used for simple anoxemia (table 3); the CO_2 content was as nearly identical as we could make it with that used for simple hypercapnia (table 4). Figures for percentile changes in respiratory minute volume are omitted because such figures, and the deductions to be drawn from them, would be entirely different depending on whether the control value was that found during quiet breathing of

TABLE 4

The effects of simple hypercapnia (inhalation of approximately 3.5 per cent CO_2 in O_2)

In all cases the same mixture was used before and after the denervation, enough having been prepared in a Douglas bag for two inhalations. The indicated changes in respiratory minute volume are measured from the period of O_2 inhalation immediately preceding this (table 2).

EXPERIMENT	ARTERIAL BLOOD						RESPIRATION					
	Sat. with O_2,* per cent		pCO_2, mm. Hg		pH_s		Normal			Denervated		
	Normal	Denervated	Normal	Denervated	Normal	Denervated	Rate	Min. vol.	Change	Rate	Min. vol.	Change
								cc.	*per cent*		*cc.*	*per cent*
1	100	100	35	27	7.38	7.43	16	6500	+109	21	9000	+77
2	100	100	38	30	7.42	7.34	10	5000	+54	14	8750	+84
3	100	100	43	37	7.32	7.36	10	4250	+37	11	6750	+69
Average, 1–3.....	100	100	39	31	7.37	7.38	12	5250	+72	15	8170	+76
4	100	100	35	34	7.43	7.42	12	5000	+67	15	6000	+72
5	100	100	34	27	7.38	7.43	8	4000	+46	10	6500	+74
6	100	100	43	37	7.36	7.40	7	3250	+45	9	4750	+73
7	100	100	40	35	7.22	7.19	6	3500	+46	8	4400	+24
8	100	100	47	35	7.24	7.30	9	7250	+91	14	11000	+87
9	100	100	44	40	7.30	7.32	8	8000	+93	9	10000	+86
10	100	100	46	42	7.31	7.27	32	7000	+54	22	8250	+80
11	100	100	50	44	7.29	7.29	8	3750	+47	12	6000	+41
12	100	100	59	50	7.17	7.20	11	7250	+61	15	11250	+55
13	100	100	57	45	7.23	7.17	16	5750	+53	16	9000	+72
14	100	100	47	40	7.23	7.21	11	7000	+84	14	11000	+91
Average, 4–14.....	100	100	45	39	7.29	7.29	12	5610	+63	13	8000	+69

* The O_2 content of most of these blood samples was greater than that corresponding with 100 per cent saturation, the excess being presumably due to O_2 in physical solution. We have been unable to find an acceptable method for calculating arterial pO_2 under such circumstances and have therefore omitted this column from this table.

room air or that existing during the period of simple anoxemia that immediately preceded this inhalation. We could find no compelling reason for preferring one of these control values to the other; since the important factor is the actual level of pulmonary ventilation we have simply indicated this. To permit ready estimation of the influence of anoxia on the response to inhalation of 3.5 per cent CO_2, the effects of the latter inhalation in high O_2 and in low O_2 are compared in the last six vertical columns of table 5; the last pair of columns summarizes these data.

TABLE 5

The effects of simultaneous anoxemia and hypercapnia (inhalation of 3.5 per cent CO_2 in 12–14 per cent O_2 in expts. 1–3, in 10–12 per cent O_2 in expts. 4–14)

The effects of this on respiratory minute volume are also shown in comparison with those of 3.5 per cent CO_2 in O_2; the last two vertical columns give the difference between the minute volume produced by this and by CO_2 in low O_2.

EXPERIMENT	ARTERIAL BLOOD								RESPIRATION				INCREASE IN RESP. MIN. VOL. (cc.) PRODUCED BY CO_2				EFFECT OF LOW O_2 ON MIN. VOL. (cc.) RESPONSE TO CO_2	
	Sat. with O_2, per cent		pO_2, mm.Hg		pCO_2, mm.Hg		pH_a		Normal		Denervated		Normal		Denervated			
	Normal	Denervated	Normal	Denervated	Normal	Denervated	Normal	Denervated	Rate	Min. vol.	Rate	Min. vol.	96.5 per cent O_2	10–12 per cent O_2	96.5 per cent O_2	12–14 per cent O_2	Normal	Denervated
										cc.		cc.						
1	92	90	61	68	25	39	7.44	7.28	24	13500	24	9000	3600	7500	3900	5000	+3900	+1100
2	90	93	53	75	31	39	7.47	7.28	10	5750	13	7250	1750	2250	4000	3750	+500	−250
3	90	90	59	61	39	34	7.36	7.36	9	5000	11	7500	1250	2750	2750	2750	+1500	0
Average, 1–3	91	91	58	68	32	37	7.42	7.31	14	8080	16	7920	2200	4160	3550	3660	+1960	+116
4	82	34	39	20	27	41	7.51	7.39	19	9250	12	3500	2000	3000	2500	1250	+1000	−1250
5	82	51	43	28	25	30	7.46	7.36	8	8250	11	5250	1250	2750	2750	1750	+1500	−1000
6	71	69	38	40	37	42	7.38	7.32	9	5500	8	3750	1000	1000	2000	1750	0	−250
7	60	72	39	49	39	34	7.22	7.20	8	4600	7	3800	1100	1200	850	1400	+100	+550
8	69	64	41	38	36	35	7.30	7.30	12	10200	10	10200	3950	4700	5100	5450	+750	+350
9	74	70	43	43	39	40	7.33	7.31	9	13000	8	10100	3850	7750	4300	6100	+3900	+1800
10	81	41	48	29	41	50	7.34	7.22	43	10800	24	4550	2450	4700	3650	1350	+2250	−2300
11	59	59	35	38	46	42	7.30	7.25	12	5600	12	5250	1200	850	1750	2250	−350	+500
12	63	33	45	25	48	58	7.26	7.17	11	7250	16	6250	2750	2250	4000	2250	−500	−1750
13	64	59	39	41	44	44	7.27	7.22	12	8250	15	6750	2000	2650	3750	3000	+650	−750
14	79	77	49	50	33	38	7.32	7.24	16	11750	13	9000	3200	5500	5250	5250	+2300	0
Average, 4–14	71	57	42	36	38	41	7.34	7.27	14	8590	12	6220	2250	3300	3230	2900	+1130	−750

The average figures which appear at the bottom of table 5 give a fair portrayal of the important trends in these animals; the first three experiments can be excluded on the ground of insufficient anoxemia to warrant

conclusions. Before chemoreceptor denervation the addition of 3.5 per cent CO_2 to 10 per cent O_2 tended to increase the O_2 content and tension of arterial blood as well as the minute volume of breathing. The actual level of pulmonary ventilation was with only two exceptions greater than that attained during inhalation of 3.5 per cent CO_2 in O_2, and this was true even with minimal anoxemia (expts. 1–3). Thus the hyperpnea of mild hypercapnia was definitely potentiated by anoxemia in these animals before chemoreceptor denervation.

After chemoreceptor denervation the results were significantly different. In the experiments (nos. 4–14) in which definite O_2-lack was present before denervation, the anoxemia tended to be more severe after denervation even though the inhaled mixture contained 12 per cent O_2 instead of 10; arterial pO_2 would have fallen more consistently and strikingly had it not been for the considerable decreases in pH which now occurred. The level of pulmonary ventilation reached during inhalation of the CO_2-O_2-N_2 mixture was less than it was before denervation in all of experiments 4–14 except one, in which the two values were the same; this level was in fact less than that reached with CO_2 in O_2 in all of these experiments except one (no. 9), in which the two figures were practically identical. This shows that the additive effects of anoxemia and hypercapnia on respiration in these animals were entirely due to chemoreceptor reflexes. As shown in the last two columns of table 5, some potentiation of the CO_2 response by anoxemia was apparent after denervation in 4 of the 11 experiments in which severe anoxemia was induced, but in only one (expt. 9) was it at all striking. Although we have no reason to doubt the validity of this particular result, it was clearly exceptional and the trend certainly was in the opposite direction, i.e., anoxemia in the absence of chemoreceptor reflexes tended to diminish the stimulant effect of CO_2 on the center. This cannot be attributed to decreased responsiveness to CO_2 *per se* because CO_2 in O_2 tended definitely to produce a higher level of pulmonary ventilation after the denervation than before it.

Our experiments have also yielded some data on the relative importance of the two major factors involved in the increased tolerance to atmospheres deficient in O_2 consequent on the addition of CO_2, viz., increased depth of breathing, and shift of the dissociation curve of oxyhemoglobin. With chemoreceptors intact the average depth of breathing during exposure to 10 per cent O_2 was increased by the addition of 3.5 per cent CO_2 from 440 cc. (table 3) to 613 cc. (table 5); simultaneously the average pH fell from 7.40 to 7.34. The result of both these influences was a rise in the average O_2 saturation of arterial blood from 68 to 71 per cent and in the average pO_2 from 36 to 42 mm. Hg. After the denervation the addition of 3.5 per cent CO_2 to 12 per cent O_2 increased the average depth of breathing from 300 to 518 cc.; average pH fell from 7.33 to 7.27, so that this factor was considerably greater than before. Nevertheless the total compensa-

tion for 12 per cent O_2 was now considerably less effective than that previously seen to 10 per cent O_2, for average saturation with O_2 increased only from 55 to 57 per cent and average pO_2 only from 33 to 36 mm. Hg. The factor responsible for the difference in pO_2 must have been the smaller depth of breathing after the denervation (518 compared with 613 cc.), which means that chemoreceptor reflexes aroused by anoxemia could not be replaced either by a greater chemical stimulation of the center (average arterial cH and pCO_2 were both greater after the denervation) or by greater displacement of the dissociation curve. Thus the reflex factor appears to have been the most important item in the defense of these animals against anoxemia even when 3.5 per cent CO_2 was added to the inspired air.

Another line of evidence leading to the same conclusion is the decrease in the ΔpO_2 (the difference between the oxygen tension in the inspired air and that in the arterial blood) attributable to chemoreceptor reflexes and to displacement of the dissociation curve. The averages of our results bearing on this point are shown in figure 1, which is so constructed that improvement in the body's compensation (which means decrease in ΔpO_2) is indicated by rise of the curves. Before chemoreceptor denervation inhalation of 10 per cent O_2(pO_2 = 71 mm. Hg) caused the ΔpO_2 to diminish from 89 to 35 mm. Hg; the change in arterial pH (from 7.31 to 7.40) caused arterial pO_2 to be 4 mm. Hg lower than it would otherwise have been at that saturation and therefore worked to the disadvantage of the organism. After denervation inhalation of 12 per cent O_2(pO_2 = 86 mm. Hg) was associated with a decrease in ΔpO_2 from 82 to 53 mm. Hg. The superiority of the one curve over the other (18 mm. Hg) is entirely referable to the chemoreceptors. When 3.5 per cent CO_2 was added before chemoreceptor denervation the ΔpO_2 diminished to 29 mm. Hg; the drop in arterial pH from 7.40 to 7.34 accounted for 4 of the 6 mm. improvement in arterial pO_2. After denervation the addition of 3.5 per cent CO_2 decreased the ΔpO_2 only from 53 to 50 mm. Hg; the part played by the chemoreceptors is indicated by the 17 mm. Hg distance between this curve and that representing the ΔpO_2 if there had been no change in pH. The part played by the action of the increased pCO_2 on the respiratory center is shown by the figures and arrows on the right side of figure 1, viz., 6 mm. Hg before denervation, 3 mm. Hg afterward; the difference between these figures can be construed as meaning either that chemoreceptor reflexes play a vital part in the response to inhalation even of 3.5 per cent CO_2, or that anoxia depresses the response of the center to CO_2, the effect being overshadowed by chemoreceptor reflexes as long as they are active. Since chemoreceptor denervation did not reduce the response of these animals to inhalation of 3.5 per cent CO_2 in O_2 (table 4), the second of these explanations seems preferable. Whether the diminished effectiveness of CO_2 in decreasing the ΔpO_2 after the denervation is referable simply to the additive

effects of anoxemia (reflex) and hypercapnia (central) on the depth of breathing (518 as compared with 613 cc. on the average), or is due to partial compensation for the central depressant effects of anoxia by strong excitatory nerve impulses from the chemoreceptors, must be determined by future investigations. At present it seems likely that both factors are concerned.

In these experiments we covered the entire range of anoxemia practicable without artificial respiration, from the mildest (expts. 1–3) to the most

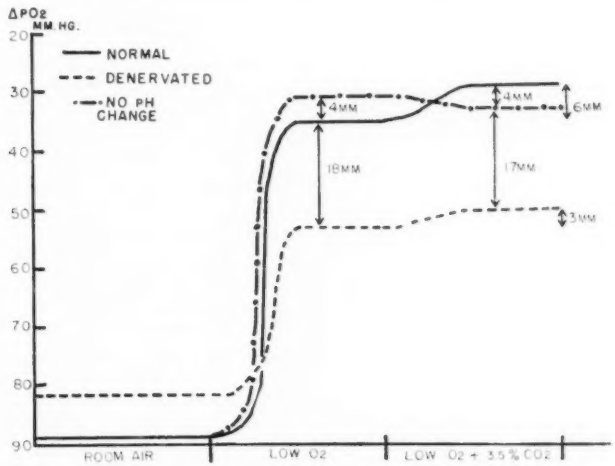

Fig. 1. Relative importance of chemoreceptor reflexes and of shifts in the dissociation curve in compensating for anoxemia. From the average results of experiments 4 to 14 (see tables). Abscissae are arbitrary units indicating change in composition of inspired air. Ordinates show ΔpO_2 (difference between pO_2 in inspired air and pO_2 in arterial blood); values for pO_2 of inspired mixtures are based on 150 mm. Hg as that for room air; values for arterial pO_2 are those given in tables 1, 3, and 5; rise in curves means diminution in ΔpO_2 and therefore better compensation, for if latter were maximal $\Delta pO_2 = 0$. The curves (other than normal) show the observations made after chemoreceptor denervation (*denervated*) and the calculated values for ΔpO_2 (i.e., the change in arterial pO_2) if arterial pH had remained at its previous level (*no pH change*). The numbers with the arrows on the right side indicate the increase in arterial pO_2 produced by the addition of 3.5 per cent CO_2 to the low-oxygen mixture; the other numbers with arrows show the distance (in mm. Hg) between the corresponding curves.

severe. In a number of experiments 10 per cent O_2 or less was used after the denervation; in all of these progressive and quite rapid failure of respiration and circulation occurred, whether CO_2 was added or not, and a steady state was never attained. This undoubtedly was because the depressant effect of severe anoxia on the respiratory and vasomotor centers

and on the myocardium was no longer opposed by chemoreceptor reflexes aroused by anoxemia; the other factors (increase in pCO_2 and decrease in pH) must have been augmented even more markedly in these cases than in those shown in table 5 and the end result again indicates the dominant importance of chemoreceptor reflexes in compensating for atmospheres deficient in O_2. We therefore believe that the conclusions mentioned above are applicable to all grades of anoxemia, subject to the limitations of these experiments (lightly anesthetized dogs with carotid pressoreceptors and the entire aortic reflex zone inactivated). The possible influence of the anesthetic on these results is now under investigation.

4. Other observations. a. *The influence of chemoreceptor denervation on respiratory activity during quiet breathing of room air.* Data bearing on this are shown in table 1. Obviously they give no indication that chemoreceptor reflexes had played an indispensable (or indeed detectable) part in the maintenance of pulmonary ventilation in these animals at rest. Actually there was an increase in the minute volume after the denervation in all but one of the experiments; this may have been due to irritation of nerve fibers as a result of the denervation procedures or to partial recovery from the effects of the narcotic injected some two hours previously, or perhaps to both. The observed changes in the blood were such as would be expected if the respiratory center had become more excitable or was being influenced more by afferent excitatory nerve impulses; the increased breathing clearly cannot be attributed to a coincidental increase in the chemical stimulus and the conclusion that chemoreceptor reflexes had not been influencing the center to an important extent is further supported by the tendency of arterial pCO_2 to be lower after the denervation than before. Generalizations concerning an important rôle of chemoreceptor reflexes in the maintenance of pulmonary ventilation under normal resting conditions therefore are not justified by existing evidence.

b. *The response to sodium cyanide.* This drug was routinely injected intravenously in the same dosage (0.2 to 0.4 mgm. per kilo) before and after the denervation, the first injection to test the reactivity of the chemoreceptor system, the second to test the completeness of the denervation. Since no animal was used in which the first injection failed to stimulate breathing considerably there was no case among the 14 in which hyperpnea was lacking. Its intensity was greater than that even of the most effective gas mixture (CO_2-O_2-N_2), ranging from an increase of 109 per cent to one of 400 per cent in respiratory minute volume. Blood pressure also rose, without exception, though often slightly; the observed rises ranged from 4 to 74 mm. Hg. After the denervation there was a pure depression in respiratory minute volume following the same injection in all but 3; in 2 there was no change, in the other the increase was from 5000 to 5200 cc. per minute (4 per cent), which can scarcely be called significant. Blood pressure fell in all cases but one, in which it was unchanged; the falls ranged from 10 to 122 mm. Hg. These results show clearly that the stimulant effects of NaCN, at least in the doses employed and under the conditions of these experiments, are entirely due to chemoreceptor reflexes. In a few cases we gave a larger dose (1.0 mgm. per kilo); this caused alarming depression of circulation after the denervation with no stimulation of respiration.

In view of Dautrebande's claim (5) that aortic reflexes play no part in chemoreceptor phenomena it may be well to record the results obtained in one of these animals leading to the conclusion that fibers from the aortic body were included with the vagus and were not limited to the depressor filament (p. 2 above). In

TABLE 6

The effects on blood-pressure (mean pressure in a femoral artery as recorded by a mercury manometer)

The indicated changes are in millimeters of mercury

EXPERIMENT	CHEMORECEPTOR REFLEXES ACTIVE												CHEMORECEPTOR REFLEXES INACTIVE											
	Room air to 100 per cent O_2			100 per cent O_2 to $O_2 + CO_2$			Room air to low O_2			Low O_2 to low $O_2 + CO_2$			Room air to 100 per cent O_2			100 per cent O_2 to $O_2 + CO_2$			Room air to low O_2			Low O_2 to low $O_2 + CO_2$		
	From	To	Diff.	From	To	Diff.	From	To	Diff.	From	To	Diff.	From	To	Diff.	From	To	Diff.	From	To	Diff.	From	To	Diff.
1	190	190	0	190	190	0	168	158	−10	158	130	−28	166	168	+2	168	196	+18	158	148	−10	148	138	−10
2	180	188	+8	188	184	−4	176	144	−22	144	140	−4	130	140	+10	140	150	+10	130	124	−6	124	130	+6
3	200	202	+2	202	198	−4	204	196	−8	196	200	+4	210	230	+20	230	228	−2	190	194	+4	194	184	−10
Average, 1–3	190	193	+3	193	191	−2	183	166	−17	166	157	−9	169	179	+10	179	191	+12	159	155	−4	155	151	−4
4	174	184	+10	184	178	−6	140	116	−24	116	116	0	154	154	0	154	148	−6	142	104	−38	104	60	−44
5	184	172	−12	172	150	−22	146	150	+4	150	142	−8	190	196	+6	196	190	−6	174	152	−22	152	140	−12
6	218	206	−12	206	220	+14	200	194	−6	194	190	−4	210	226	+16	226	228	+2	210	194	−16	194	166	−28
7	200	188	−12	188	192	+4	138	124	−14	124	110	−14	106	118	+12	118	120	+2	104	90	−14	90	94	+4
8	220	226	+6	226	clot		206	196	−10	196	184	−12	164	184	+20	184	196	+12	170	156	−14	156	148	−8
9	258	250	−8	250	258	+8	230	212	−18	212	218	+6	210	230	+20	230	228	−2	164	132	−32	132	136	+4
10	110	120	+10	120	130	+10	134	142	+8	142	132	−10	174	180	+6	180	180	0	146	82	−64	82	78	−4
11	190	196	+6	196	196	0	172	160	−12	160	154	−6	168	170	+2	170	162	−8	170	112	−58	112	106	−6
12	140	162	+22	162	150	−12	156	136	−20	136	118	−18	194	184	−10	184	clot		106	62	−44	62	68	+6
13	222	218	−4	218	206	−12	174	140	−34	140	146	+6	158	146	−12	146	140	−6	116	90	−26	90	106	+16
14	134	146	+12	146	132	−14	100	80	−20	80	80	0	116	122	+6	122	122	0	104	76	−28	76	94	+18
Average, 4–14	187	196	+10	197	181	−16	164	150	−14	150	144	−6	167	174	+7	174	171	−3	146	114	−32	114	110	−4

this animal the right vagodepressor nerve and a group of fibers identified by electrical stimulation as the left depressor were cut at the start; the left vagus was intact. Cyanide (0.4 mgm. per kilo intravenously) increased respiratory minute volume from 5000 to 12,000 cc. (140 per cent) and blood pressure from 138 to 160 mm. Hg before carotid denervation. After the latter operation the same dose increased breathing from 4900 to 8000 cc. per minute (63 per cent) and blood pressure from 178 to 194 mm. Hg. The left vagus was then cut and the injection repeated; breathing now decreased from 4500 to 4000 cc. per minute (11 per cent) and blood pressure fell from 202 to 80 mm. Hg. This, together with the data presented by Comroe (4), should suffice to disprove Dautrebande's contention.

c. *The effects on blood pressure.* The behavior of mean femoral blood pressure under the varying conditions of these experiments is summarized in table 6. We were unable to find any consistent evidence of a synergistic effect of anoxemia and hypercapnia on the vasomotor center (i.e., after chemoreceptor denervation) and our results therefore differ materially from those reported by Gellhorn and Lambert (11). As a matter of fact the only definite tendency shown by the circulations of our animals during anoxemia, hypercapnia, or the combination of the two, was toward a depression during the anoxemic periods, and this was distinctly greater after the denervation than before. Other distinct tendencies were toward a rise in blood pressure during O_2 inhalation after the denervation (due, we believe, to an improvement in functional capacity of the vasomotor neurons and the myocardial cells similar to that postulated on page 7 to explain the simultaneous increase in respiration) and toward a rise in pressure when the carotids were denervated. The latter is noteworthy because it is scarcely attributable to removal of pressoreceptor activity from the carotid sinuses, since these must have been completely inactivated by the ligation-collapse technic used for the initial denervation. Yet in 9 of the 14 experiments pressure rose distinctly when the carotid reflex zones were ligated and their attachments cut. Since the cerebral circulation was not altered by this procedure (p. 2) the only explanation for the rise in pressure is that enough pressoreceptors must have been present in the external carotid region to exert a distinct physiological effect. This is in accord with the results of a long series of unsuccessful attempts at recording action potentials from the carotid chemoreceptors of dogs without contamination by impulses from pressoreceptors in this region (see 19, p. 117). The point is important in the evaluation of studies of action potentials in the sinus nerve after denervation of the carotid sinus pressoreceptors; if such impulses do not represent chemoreceptor activity exclusively, they are not capable of yielding valid evidence on questions involving quantitative characteristics of the chemoreceptors, as they have been assumed to be.

SUMMARY AND CONCLUSIONS

1. In 14 lightly anesthetized dogs with aortic chemoreceptors and carotid pressoreceptors inactivated, the effects of inhalation of the following gas mixtures on pulmonary ventilation and on the gas tensions and pH of arterial blood were studied before and after denervation of the carotid chemoreceptors: room air, 100 per cent O_2, O_2 + 3.5 per cent CO_2, 10 to 14 per cent O_2 in N_2, 10–14 per cent O_2 + 3.5 per cent CO_2.

2. Inhalation of O_2 while the chemoreceptors were active caused transitory respiratory depression in some cases, but in only one did this persist as long as 5 minutes; in one other, stimulation of breathing occurred and

in the rest there was no significant effect; the animal that showed prolonged depression of breathing had the lowest arterial oxygen tension (50 mm. Hg) before O_2 was given. After chemoreceptor denervation O_2 inhalation quite regularly caused stimulation of respiration (and circulation); this phenomenon therefore is not related to chemoreceptor reflexes while depression of breathing on inhalation of O_2 certainly is. Pulmonary ventilation during quiet breathing of room air was not diminished by the denervation, indicating that no indispensable reflex activity was maintained during eupnea in these animals.

3. CO_2 in O_2 stimulated breathing at least as much after chemoreceptor denervation as before. There was no sign that chemoreceptor reflexes played any measurable part in the animals' responses to inhalation of CO_2 in this strength.

4. When the chemoreceptors were intact mild anoxemia (12 per cent O_2) caused slight if any respiratory stimulation, but more severe anoxemia (10 per cent O_2) had more marked effects; after denervation depression was the usual result, showing that the hyperpnea of anoxemia is entirely reflex in origin. The degree of anoxemia produced by a given O_2-N_2 mixture was decidedly greater after the denervation than before. The threshold of the chemoreceptors to anoxemia lay at an arterial O_2 saturation of about 85 per cent and an arterial pO_2 of 50 to 55 mm. Hg.

5. The addition of 3.5 per cent CO_2 to 10 per cent O_2 before chemoreceptor denervation increased the O_2 content and tension of arterial blood as well as pulmonary ventilation; the latter was nearly always greater now than it was during inhalation of 3.5 per cent CO_2 in O_2. After chemoreceptor denervation inhalation of 3.5 per cent CO_2 in 12 per cent O_2 did not bring arterial O_2 content and tension even as high as it was with CO_2 in 10 per cent O_2 before denervation and pulmonary ventilation was not as great as it was with CO_2 in O_2. The additive effects of anoxemia and hypercapnia on respiratory minute volume were therefore due entirely to chemoreceptor reflexes aroused by the former; the only direct effect of anoxia on the response of the center to CO_2 was a depressant one. Reasons are presented for believing that increased depth of respiration referable to the chemoreceptors is more important than direct stimulation of the center by CO_2 or a shift in the dissociation curve of oxyhemoglobin in explaining the rise in arterial pO_2 consequent on the addition of CO_2 to an inspired mixture low in O_2.

6. The effects of these procedures on blood pressure were not significant, with two exceptions, *viz.*, rise in pressure on carotid denervation and further rise on inhalation of O_2 after the denervation. The former is attributed to the presence of pressoreceptors in the external carotid distribution, the latter to improvement in the functional capacity of the vasomotor center and myocardium as well as the respiratory center when arterial O_2 tension is raised after the animal had been exposed to anoxemia.

7. The stimulant effects of intravenous injections of sodium cyanide on respiration and circulation, which were very intense before chemoreceptor denervation, gave way to no effect or to pure depression afterward; these effects were therefore entirely reflex.

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THE EFFECT OF VASOCONSTRICTOR SUBSTANCES IN SHED BLOOD ON PERFUSED ORGANS[1]

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One of the chief difficulties in the perfusion of surviving organs with blood has been its vasoconstrictor effect reported by many investigators (reviewed by Amberson (1) and Gaddum (2)). The constrictor substances or vasotonins originate in blood immediately after its withdrawal from the organism (3) and are present in defibrinated (3), heparinized blood (3), and blood serum (4). Only after Starling's work with the heart lung preparation (5) did the rôle of the perfused lung in eliminating these vasotonins from the blood become apparent. More recently it has been shown by Heymans and co-workers (6) and by Bayliss and Ogden (3) that the constrictor effect of shed blood on perfused organs is temporarily abolished by the addition of ergot preparations to the perfusion fluid. The present paper deals with the influence of vasotonins on the oxygen consumption of the perfused submaxillary gland and kidney of the cat, with the rôle played by the lung and other organs in eliminating these constrictor substances from the perfusate and with the effect of various doses of ergotoxine on the blood flow of the perfused kidney.

Cats anesthetized with nembutal (0.6 cc. per kgm. body weight, injected intraperitoneally) were used in all experiments. One hundred and twenty cubic centimeters of defibrinated blood were used as perfusate. The perfusion system consisted of two parts: one the perfusion pump, the other a unit for the creation and maintenance of a constant pulsating pressure consisting of an oil flask and a rotating valve described by Lindbergh (7).

The perfusion pump, designed for the simultaneous perfusion of two organs, is made of pyrex glass. It consists of four chambers: a pressure chamber, a reservoir chamber and two organ chambers (fig. 6). Stopcocks, acting as valves, separate the pressure chamber from the reservoir chamber and the reservoir chamber from the two organ chambers.

The valve action of the stopcocks *A* and *B* is controlled by a motor-

[1] Part of this paper was presented before the American Physiological Society, Proc., This Journal, 1939.

driven mechanism, by means of which the various passages in the pump are opened and closed at regular intervals. The timing of the intervals can be varied between 5 and 25 cycles per minute.

In the first portion of the perfusion cycle, the organ chambers are in communication with the equalization chamber; there is no connection between the equalization chamber and the pressure chamber. The equalization chamber, however, is open to the atmosphere through an aperture in the stopcock *A*, and the perfusate flows from the organ chambers into the equalization chamber. The flow is allowed to continue for a predetermined period, then it is stopped by rotating stopcock *A*, which closes the passage between the organ chambers and the equalization chamber. Simultaneously the connection of the equalization chamber with the atmosphere through stopcock *A* is closed and a passage is opened to the one-way valve assembly of the oil flask, thus introducing pressure into the equalization chamber.

The two stopcocks are so arranged that the rotation of stopcock *A* which completes the first portion of the perfusion cycle is accompanied by a similar rotation of stopcock *B* which opens the passage between the pressure and the equalization chambers. Since now both pressure and equalization chambers are connected to the one-way valve assembly of the oil flask, the pressure within the two chambers becomes the same, and the perfusate flows from the equalization into the pressure chamber by gravity. This flow continues until the equalization chamber is empty, when the stopcocks are automatically adjusted to their original positions.

The action of the equalization chamber is similar to that of an air lock, the pulsating pressure within the pressure chamber being constantly maintained. The arteries of the organs undergoing perfusion are connected with cannulas from the pressure chamber, the pressure in the artery of the organ being the same as that within the pressure chamber. As in the Lindbergh pump, the pulse rate and pressure are variable. Since the organ chambers are under atmospheric pressure, sealing of the pump is unnecessary. This reduces the period during which the organs are separated from their blood supply to less than thirty seconds.

The organs were excised while the anesthetized animal was alive. In order to avoid clotting in situ, 10 mgm. of heparin[2] were injected intravenously. The kidney was excised according to the technique of Steggerda, Essex and Mann (8), which has the advantage of preserving normal blood supply to the organ during the operation. The lung was perfused through the pulmonary artery, the submaxillary gland through a cannula inserted into the external carotid artery. The blood flow of the submaxillary gland in vivo was measured according to the method of Barcroft and Piper (9); that of the kidney in vivo by means of a cannula inserted

[2] Connaught Laboratories, Toronto, Canada.

into the renal vein. Blood samples in vitro were taken from the cannula connecting the organ chambers with the pressure chambers and from the venous blood dripping from the organ. The Van Slyke manometric method (10) was used for the determination of the oxygen content of the arterial and venous blood. The organs were perfused at a temperature of 38°C.

Oxygen consumption and rate of flow of the perfused submaxillary gland and the kidney. The effect of vasotonins on the metabolism of the submaxillary gland was investigated in twenty-five experiments in which the oxygen consumption and the blood flow of the organs were measured in situ and after their transplantation into the perfusion pump. The lung was not included in the perfusion circuit in these experiments. The oxygen consumption of the gland in situ ranged from 0.01 to 0.03 cc. of oxygen/gram/minute, values which correspond to those found by Barcroft and Piper (9). In the perfusion apparatus the values for the oxygen consumption lay between 0.002 and 0.009 cc. of oxygen/gram/minute (fig. 1). Most of this fall in the oxygen consumption from animal to pump was caused by vasoconstriction in the perfused organ, since the rate of venous outflow of the gland fell from an average of 0.5 cc. of blood/gram/minute to values lying between 0.2 to 0.35 in the perfusion apparatus (fig. 2).

In twenty-five experiments on the kidney the fall in the oxygen consumption and the rate of flow from animal to pump was found to be even more marked (fig. 1). The organ in vivo consumed from 0.05 to 0.08 cc. of oxygen/gram/minute; in the perfusion apparatus only from 0.003 to 0.06 cc. of oxygen/gram/minute. The rate of flow dropped an average of 0.7 cc./gram/minute after transplantation of the organ into the perfusion apparatus (fig. 2).

The effect of the perfused lung. In twelve experiments blood was circulated through the ventilated isolated lung for thirty minutes before the kidney or the salivary gland was connected with the perfusion pump. Parallel perfusion of the lung with either the kidney or the salivary gland was then continued for two hours. The influence of the lung on the rate of flow of the kidney and the submaxillary gland is shown in figure 3. The inclusion of the lung in the perfusion circuit reduced the difference between the renal blood flow in vitro and in vivo to less than 0.05 cc. of blood/gram/minute. There was no difference between the rate of flow of the perfused submaxillary gland and that of the organ in vivo after inclusion of the lung (fig. 3).

In order to investigate whether the effect of the lung was connected with its respiratory function, the kidney was perfused in five experiments with the ventilated and in four with the unventilated lung. Kidney perfusion was started five minutes before inclusion of the lung. As soon as the lung was connected in the circuit the renal blood flow rose, reaching five times its previous value after thirty minutes. The unventilated lung

did not differ from the ventilated lung in its effect upon the renal blood flow (fig. 4, A and B). The injection of 30 cc. of freshly defibrinated blood into the perfusion fluid caused an immediate fall in blood flow, indicating the addition of new vasotonins to the perfusate (fig. 4A). With the lung in the circuit, however, the constrictor effect disappeared quickly.

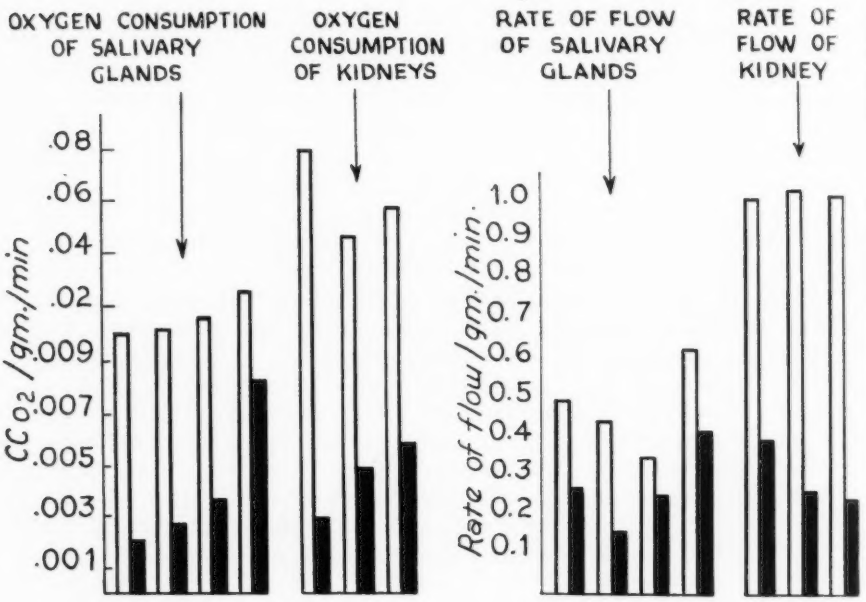

Fig. 1 Fig. 2

Fig. 1. Shows the oxygen consumption of the submaxillary gland and the kidney of the cat in the animal and after transplantation of the organs into the perfusion apparatus. The white column represents the oxygen consumption of the organs in the animal, the black column indicates the values found in the perfusion apparatus.

Fig. 2. Demonstrates the rate of flow of the submaxillary gland and the kidney of the cat in the animal and after transplantation of the organs into the perfusion apparatus. White column represents the rate of blood flow of the organs in the animal, black column shows values found in the perfusion apparatus.

Effect of liver, spleen and kidney. It has been demonstrated that the property of removing vasoconstrictor substances from shed blood is not limited to the lung. Bornstein (11) has shown that the liver eliminates vasotonins from its perfusates. Experiments were therefore performed to reëxamine the action of that organ and to extend the investigation to the perfused spleen.

Parallel perfusions of the kidney with the liver or with the spleen were

undertaken. As in the previous experiments, the rate of venous outflow from the kidney served as an indicator of the constrictor properties of the perfusate. The experiments demonstrated that the liver as well as the spleen removed vasotonins from the perfusate at rates equaling that of the lung.

The rôle of the kidney in the elimination of vasotonins has been investigated by Bayliss and Ogden (3). Those investigators, perfusing that organ without the inclusion of the lung, found that complete detoxication of the blood was never achieved by the kidney alone. Experiments in

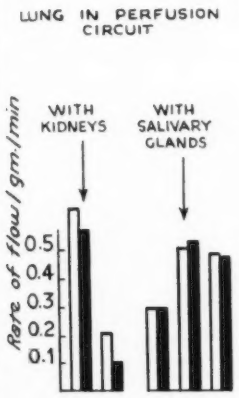

Fig. 3

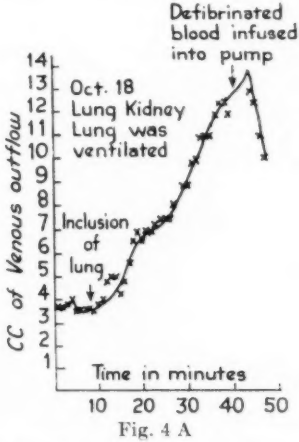

Fig. 4 A

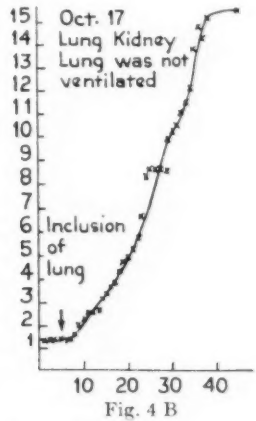

Fig. 4 B

Fig. 3. Shows the blood flow of the kidney and the submaxillary gland of the cat in the animal and in the perfusion apparatus, after the lung had been included into the perfusion circuit. White column represents blood flow in the animal, black column indicates values found in the perfusion apparatus.

Fig. 4 A and B. Demonstrates the effect of the perfused lung on the blood flow of the isolated kidney. In A the lung is ventilated. The inclusion of both ventilated and unventilated lung causes an increase in the renal blood flow.

which the isolated kidney alone was perfused with defibrinated blood, confirmed this result. Twenty minutes passed before any effect on the renal blood flow was noticeable. After forty-five minutes of kidney perfusion, the blood flow through that organ had increased only from 3.5 to 5 cc./minute.

The effect of ergotoxine. Since Heymans and co-workers (6) and Bayliss and Ogden (3) have shown that the action of vasotonins was inhibited by ergot preparations, experiments were performed to study the influence of various doses of ergotoxine[3] on the rate of venous outflow of kidneys per-

[3] Ergotoxine Ethanesulphonate, Burroughs Wellcome & Company, New York.

fused with defibrinated blood. Doses of ergotoxine ranging from 0.01 mgm. to 5 mgm. were injected into the perfusate and their effect on the venous outflow from the kidney recorded.

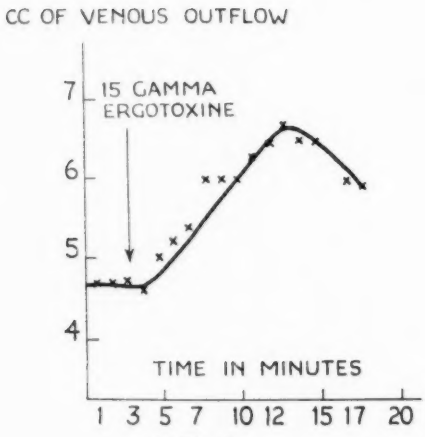

Fig. 5

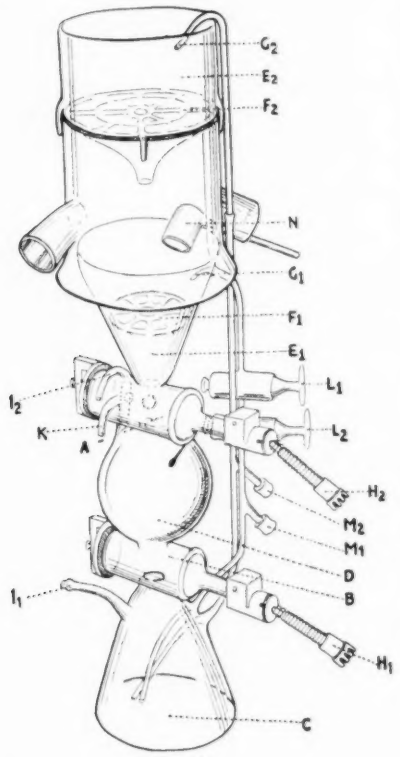

Fig. 6

Fig. 5. Demonstrates the effect of 15 γ of ergotoxine ethanesulphonate on the blood flow of the perfused kidney. The vasodilator effect of the drug lasts ten minutes. After that period the renal blood flow decreases.

Fig. 6. Perfusion pump. *A*, stopcock separating the two organ chambers from the equalization chamber; *B*, stopcock separating pressure chamber from equalization chamber; *C*, pressure chamber; *D*, equalization chamber; E_1 and E_2, the two organ chambers; F_1 and F_2, glass grids on which the organs rest; G_1 and G_2, glass cannulas leading to the arteries of the perfused organs; H_1 and H_2, flexible connections to the motor drive turning the stopcocks; I_1 and I_2, pressure inlets from oilflask; *K*, equalization chamber vent; L_1 and L_2, stopcocks for regulating the arterial blood flow to the organs; M_1 and M_2, injection caps; *N*, sampling cannula for the collection of venous blood from the perfused organ.

The action of small doses of the drug differed from that of larger ones. The injection of 15 γ of ergotoxine produced in three experiments a slight

increase in the blood flow, starting immediately after the injection and lasting for ten minutes. After that period the action of vasotonins became evident again and the blood flow decreased (fig. 5).

The injection of larger doses of the drug resulted in immediate vasoconstriction. In one instance 3 mgm. of ergotoxine caused a decrease in blood flow from 4.6 to 2 cc. per minute; after this period of constriction, however, the rate of flow increased steadily, amounting to 10 cc./minute after thirty minutes; no return to the original low blood flow was noticeable for the duration of the experiment.

Discussion. The action of vasoconstrictor substances in shed blood on perfused organs is demonstrated in experiments in which the metabolism and the rate of flow of the kidney and the submaxillary gland are measured in the organism and after transfer of these organs to a perfusion apparatus. The values for the rate of flow and the oxygen consumption of those organs are much lower in the perfusion apparatus than in the whole animal (figs. 1 and 2). The metabolism of the kidney is reduced from an average of 0.05 to 0.004 cc. of oxygen/gram/minute, that of the submaxillary gland from an average of 0.02 cc. to 0.005 cc. of oxygen/gram/minute. This fall in the oxygen consumption demonstrates the difficulty arising from the use of shed blood as perfusate.

When the lung is included in the perfusion circuit, the rate of flow of the kidney and salivary gland attain the values found in the whole animal. The oxygen consumption also becomes normal, indicating that its previous low value was due to vasoconstriction. The effect of the lung in removing the vasotonins from its perfusate is not connected with the respiratory function of that organ. This is demonstrated in experiments in which it is shown that both ventilated and unventilated lungs have the same effect on the constrictor properties of the perfusate.

The isolated lung, however, is not the only organ to remove vasotonins from the perfusate. The inclusion of either the isolated liver or the spleen in the perfusion circuit is as effective in raising the blood flow of the kidney as the inclusion of the lung. This confirms Bornstein's (11) observation that shed blood is detoxicated by its passage through the liver. The perfused kidney without the inclusion of other organs in the perfusion circuit has little influence on the constrictor properties of the blood. Since Budelmann (12) has shown that the isolated placenta has no effect upon the constrictor properties of its perfusate, it can be concluded that the elimination of vasotonins is limited to a number of organs, such as the spleen, the lung and the liver.

The addition of ergotoxine to the perfusion fluid of isolated kidneys furnishes another means of reducing vasoconstriction in that organ. The injection of 0.02 mgm. of ergotoxine produces slight vasodilatation lasting, however, for only ten minutes. This is in agreement with the findings of Heymans and co-workers (6), who noticed only short dilator effects

following the addition of the drug to the perfusate. The injection of large doses of ergotoxine results, however, in immediate vasoconstriction. In one instance 3 mgm. of ergotoxine caused a decrease in blood flow from the kidney from 4.6 to 2 cc. per minute. After this period of constriction, however, the blood flow increases steadily approaching values found in the intact animal, and shows no tendency to return to its original low level in the course of experiments lasting for two hours.

SUMMARY

The constrictor properties of defibrinated blood are demonstrated by comparing the oxygen consumption and the blood flow of the kidney and the submaxillary gland of the cat in vivo with the oxygen consumption and the blood flow of the same organs perfused with blood in a perfusion apparatus (figs. 1 and 2).

The oxygen consumption and the blood flow of the perfused submaxillary gland fell one-half, that of the kidney to one-tenth of the values found for the organs in the animal.

Inclusion of the lung, liver or spleen in the perfusion circuit raises the blood flow of the perfused kidney and salivary gland. The action of the lung is not due to processes connected with its ventilation.

The injection of large doses of ergotoxine into the kidney perfusate causes a decrease followed by an increase in the renal blood flow lasting for several hours.

Sincere appreciation is expressed to Mr. F. Dowd for his assistance in the design and construction of the perfusion pump.

The glass part of the apparatus was blown by O. Hopf, New York City.

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THE NUTRITIONAL VALUE OF SOME COMMON CARBOHYDRATES, FATS, AND PROTEINS STUDIED IN RATS BY THE SINGLE FOOD CHOICE METHOD

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In these experiments rats were kept on a diet which contained only one of the three main foodstuffs—that is, either a carbohydrate, a fat or a protein—whenever possible in purified form. Records were made of the survival time, body weight, food and water intake, spontaneous activity and vaginal smears. The survival times for a few of the carbohydrates, fats and proteins were reported as a part of a study on the growth and reproduction of rats kept on a self-selection diet (Richter, Holt and Barelare, 1938).

METHODS. The rats were kept separately in cages which contained a revolving drum with a cyclometer and a living compartment with a food cup and either one or two graduated inverted water bottles. The living compartment was made of wire cloth with a sufficiently large mesh to permit the feces to drop through freely to pans placed about 2 inches below the bottom of the cage. Solid feces in the revolving drum fell to the underlying pans through the $\frac{1}{4}$ to $\frac{1}{2}$ inch space between the drum and the central partition.

Rats, which had been on the standard McCollum diet since weaning, were placed in the cages at an average age of 50 days and kept on this diet for about 14 days. The McCollum diet was then replaced by a single food, given in as pure a form as was obtainable. Tap water was supplied ad libitum.

Daily records were made of food and water intake, activity and of the vaginal smears; the animals were weighed weekly.

Since animals on these restricted diets have a greatly altered metabolism and are less able to withstand rapid changes in temperature than normals, special precautions were taken to keep the room temperature as nearly constant as possible (78° to 82°F.). The animals had no access to any other foodstuff or substance that could be eaten, such as sawdust or nesting paper.

The carbohydrates—dextrose (C.P., anhydrous), starch (soluble, potato,

Merck), maltose (C.P., hydrate, Pfanstiehl), sucrose (U.S.P.), levulose (C.P., special crystal, Pfanstiehl), lactose (U.S.P.) and galactose (C.P., Pfanstiehl)—were offered in the regular food cups in granulated or powdered form. The fats—olive oil (Laco), wheat germ oil (Huisking), peanut oil (Tunley), cod liver oil (U.S.P., Mead Johnson), perilla oil (Eimer and Amend) and glycerine (U.S.P., Proctor and Gamble)—were offered in fluid form in the inverted bottles; butter (saltless), lard and hydrogenated cotton seed oil (Crisco) were offered in solid form in the regular food cups. The proteins—desiccated blood fibrin (Sheffield), casein (fat and water soluble, vitamin free), three brands—Sealtest (Sealtest Laboratories), Labco (Bor-

TABLE 1

Survival time (in days)

CARBOHYDRATES				FATS				PROTEINS			
	Rats	Survival time	Average		Rats	Survival time	Average		Rats	Survival time	Average
Dextrose	4	49, 58, 60, 62	57	Butter	4	50, 51, 55, 57	53	92-Z casein digest	6	17, 25, 33, 44, 54, 107	47
Maltose	4	34, 42, 47, 52	44	Olive oil	4	30, 39, 49, 69	47	Casein (Sealtest)	3	27, 39, 47	38
Sucrose	4	35, 44, 45, 45	42	Lard	4	17, 30, 31, 32	28	Casein (McCollum)	7	20, 21, 24, 27, 32, 45, 53	32
Starch	4	21, 27, 27, 37	28	Wheat germ oil	4	20, 23, 24, 28	24	Des. blood fibrin	4	23, 29, 30, 32	29
Levulose	4	18, 18, 18, 19	18	Cod liver oil	4	10, 20, 23, 27	20	Casein (Labco)	3	20, 27, 33	27
Lactose	4	5, 5, 8, 9	7	Crisco	4	16, 18, 18, 21	18	89-7 casein digest	4	20, 21, 23, 30	24
Galactose	4	5, 5, 7, 9	7	Peanut oil	4	10, 17, 18, 23	17	Solid hemoglobin	3	20, 22, 25	22
				Perilla oil	4	3, 6, 12, 24	11	Gelatin	4	10, 11, 11, 13	11
				Glycerine	3	8, 9, 13	10	Lactalbumin	4	3, 6, 10, 14	8
Control, no food	11	3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 6	4					Zein	4	4, 4, 4, 6	5

den Company), a casein purified and autoclaved by Dr. E. V. McCollum—and casein digests, 89-7, acidified, and 92-Z, enzyme treated (Mead Johnson), zein (Mazein, Corn Products Refining Company), and gelatin (Atlantic Gelatin Company)—were all offered in solid form.

Groups composed usually of four animals were used for each substance. For control eleven rats were kept without any food at all, but with free access to tap water. One hundred and sixteen rats were used in these experiments, most of them females.

RESULTS. *Survival time.* Table 1 summarizes the effect on length of life. It gives the number of rats in each group, the individual survival times in days, and the group average for each of the groups on the various carbohydrates, fats and proteins. The survival times of the control group

of 11 rats which had no food at all averaged 4 days and had a range of from 3 to 6 days.

Carbohydrates. The rats lived 57 days on dextrose, 44 days on maltose and 42 days on sucrose. Those given starch, levulose, lactose and galactose were much shorter lived (28, 18, 7 and 7 days respectively). The rats on the last two carbohydrates lived only slightly longer than the starved controls.

Fats. The rats on saltless butter lived 53 days, and those on olive oil lived next longest (47 days). The animals on lard, wheat germ oil, cod liver oil, Crisco, peanut oil and perilla oil survived for much shorter times (28, 24, 20, 18, 17 and 11 days respectively). On glycerine alone their survival times averaged 10 days, over twice as long as without any food at all.

Proteins. The rats lived longest on the pancreatic enzyme casein digest, 92-Z (Mead Johnson), 47 days; next longest on casein (Sealtest) and McCollum casein, 38 and 32 days respectively. The rats on desiccated blood fibrin lived next longest, 29 days, followed by casein (Labco), 27 days; the casein acid digest, 89-7 (Mead Johnson), 24 days; solid hemoglobin, 22 days; gelatin, 11 days; lactalbumin, 8 days; and zein, 5 days.

Compared with the carbohydrates and fats, the proteins gave less consistent survival times. For example, the survival times of the rats on the 92-Z casein digest averaged 47 days, but ranged from 17 to 107 days; and on the McCollum casein they averaged 32 days and ranged from 20 to 53 days.

The rats lived longer on the carbohydrate, dextrose (57 days), than on any of the other single foods; next longest on the fat, butter (53 days); next longest on the fat, olive oil (47 days), and the protein, casein digest 92-Z (47 days), and on the carbohydrates, maltose (44 days) and sucrose (42 days).

Body weight. After starting on the single food diets all of the rats began to lose weight at once. Those on the carbohydrates and fats lost weight at much the same rate, while those on the proteins lost weight during the first 10 days at a somewhat more rapid rate. Figure 1 shows typical curves for each of the three foodstuffs, using butter, maltose and Sealtest casein as examples. These three curves were chosen as examples because of the fact that the average weights at the beginning of the experiments were almost the same. The butter and maltose curves were almost identical, while the casein curve showed a sharper decrease during the first 10 days. Thereafter, however, it closely paralleled the other two curves.

Food intake. From the point of view of our self-selection studies we were particularly interested to know how much of each of the different single foods the rats would eat, how the intake of the single foods compared with the previous intake of McCollum diet, and whether the intake bore any

relationship to the length of time that the rats survived. Table 2 summarizes the results. It gives the average daily food intake in grams and calories for the first 10 days on the single food diet. The last column shows

TABLE 2

*Average daily food intake for 10 days on McCollum diet and first 10 days on single food**

	MCCOLLUM DIET	SINGLE FOOD	MCCOLLUM DIET	SINGLE FOOD	CALORIC DECREASE
	Carbohydrates				
	grams	*grams*	*calories*	*calories*	*per cent*
Starch	13.5	11.2	54.0	44.8	15
Dextrose	13.3	10.9	53.2	43.6	18
Sucrose	13.3	8.5	53.2	34.0	36
Maltose	12.6	8.1	50.4	32.4	36
Levulose	12.9	6.6	51.6	26.4	44
Galactose	12.7	6.1	50.8	24.4	52
Lactose	12.9	2.0	51.6	8.0	84
	Fats				
Butter	13.6	4.5	54.5	40.5	26
Olive oil	14.8	3.6	59.2	32.4	45
Lard	11.3	2.8	45.2	25.2	44
Wheat germ oil	10.3	2.3	41.2	20.7	49
Crisco	11.8	2.5	47.2	22.5	52
Peanut oil	12.2	2.0	48.8	18.0	63
Cod liver oil	13.2	1.8	52.8	16.2	69
Glycerine	13.8	3.9	54.8	15.6	71
Perilla oil	12.3	1.0	49.2	9.0	82
	Proteins				
Des. blood fibrin	14.4	5.9	57.6	23.6	59
Casein (McCollum)	11.0	4.1	44.0	16.4	62
Casein (Labco)	12.8	4.6	51.2	18.4	64
Casein (Sealtest)	12.2	4.1	48.8	16.4	66
92-Z	11.8	4.2	47.2	16.8	69
89-7	13.5	4.0	54.0	16.0	70
Gelatin	11.3	3.0	45.2	12.0	73
Hemoglobin	14.9	3.8	59.6	15.2	74
Lactalbumin	12.2	2.8	48.8	11.2	77
Zein	12.8	0.25	51.2	1.0	100

* Calories were calculated as 4 per gram for carbohydrates, proteins, and glycerine and as 9 per gram for all fats except glycerine.

the per cent decrease during the first 10-day period in calories. During this 10-day period the intake of starch was the highest of any of the carbohydrates. As measured in grams, the food intake dropped from 13.5 grams on the McCollum diet to only 11.2 grams; as measured in calories,

it decreased from 54.0 to 44.8, or 15 per cent. The intake of dextrose for the 10-day period averaged 10.9 grams, or 43.6 calories, representing an 18 per cent decrease. The intakes of sucrose and maltose were almost identically the same, both showing a 36 per cent decrease. The intakes of levulose and galactose showed still greater decreases, 44 and 52 per cent respectively. The intake of lactose was the smallest, 2 grams per day, representing an 84 per cent decrease.

For this first 10-day period the intake of the fats dropped far below the previous levels for the McCollum diet. The rats ate much less of the fats than of the carbohydrates, as measured in grams. However, in calories the carbohydrate and fat intakes were very similar. The highest average daily intake of the fats (butter) was 4.5 grams, or 40.5 calories, while the highest of the carbohydrates (starch) was 11.2 grams, or 44.8 calories. After butter the rats showed the greatest appetite for olive oil. They

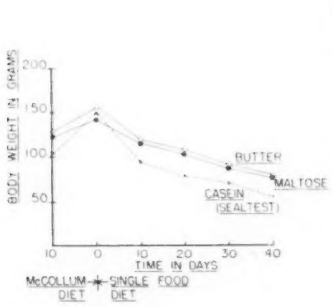

Fig. 1

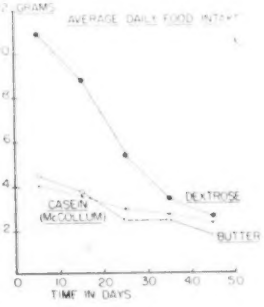

Fig. 2

showed the smallest appetite for cod liver oil (1.8 grams) and perilla oil (1.0 gram). The percentage decrease ranged from 26 for butter to 82 for perilla oil.

As measured in grams, the intake of the proteins also fell far below the intake level of the McCollum diet. During the first 10 days on the single foods, the animals on desiccated blood fibrin showed the highest intake (5.9 grams) and on zein the lowest (0.25 gram). The rats ate almost the same amounts of the 3 different caseins (4.1, 4.6, and 4.1 grams) and of the 2 digests (4.2 and 4.0 grams). They ate only small amounts of gelatin and lactalbumin (3.0 and 2.8 grams) and almost completely refused zein (0.25 gram). With the exception of zein, the percentage decrease for the different proteins fell between 59 and 77.

The intake of the carbohydrates started at rather high levels and decreased at a rapid rate, while the intake of the fats and proteins started at lower levels and decreased much less rapidly. Figure 2 gives the average

daily intake for the five 10-day periods for dextrose, butter and casein (McCollum), which are typical for their respective groups. In the 50 days the dextrose intake decreased from 10.9 to 2.8 grams; the butter intake, from 4.5 to 1.8 grams; the casein intake, from 4.0 to 2.4 grams. Thus, at the end of the 40- to 50-day period only small differences remained between the intake of the three foodstuffs.

For the carbohydrates, with the exception of starch, and for all of the fats, survival times varied directly with the average daily intake of the single foodstuffs during the first 10-day periods; but for the proteins it varied independently of the food intake. Figure 3 summarizes the results. The abscissae indicate the average survival time in days for the rats on each foodstuff; the ordinates, the average daily food intake in calories for the first 10 days, taken from table 2. With the exception of starch and galactose, all of the carbohydrates and fats fall along a line which passes through 8 calories for 7 days' survival time (lactose) to 43.5 calories for

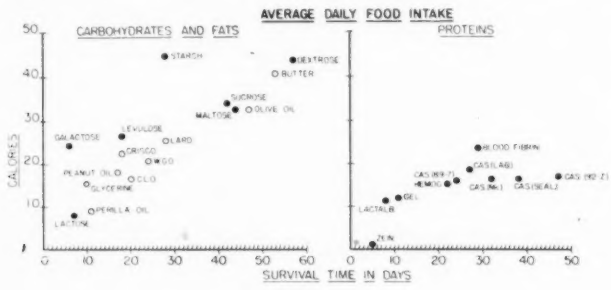

Fig. 3

57 days' survival time (dextrose). In marked contrast, the intake of the proteins, except desiccated blood fibrin and zein, averaged approximately 16 calories regardless of the survival time. Thus, on an intake of 18 calories the rats on hemoglobin lived only 22 days, while on the same intake the rats on the casein digest (92-Z) lived 47 days.

Thus, for the carbohydrates and fats the survival times seemed to depend mainly on the caloric value of the ingested foods. This relationship does not hold for the proteins. In general, the rats on proteins lived much longer than would have been expected from the caloric value of the ingested foodstuffs.

Activity. On nearly all of the single foods the rats were surprisingly active. In general, they were more active on the carbohydrates than on the fats and more active on the fats than on the proteins. Figure 4 summarizes the results. Figure 4B gives the average daily activity for the 10 days on the McCollum diet and for the 3 following 10-day periods for rats on the 3 main foods and for a control group on the McCollum diet.

It includes only animals that lived 18 days or more. Further, it is limited to animals whose average daily activity for the 10 days on the McCollum diet fell between 1000 to 8000 revolutions (17 rats on carbohydrates, 20 on fats and 11 on proteins). For the 10-day period on the McCollum diet before the start of the single food diets, the average daily activity for the controls and for the rats on the 3 different foodstuffs fell near 4000 revolutions. For the first 10 days the activity curves of the controls and of the rats on carbohydrates closely paralleled one another, while the curves for the rats on the fats and proteins showed a less marked increase in activity.

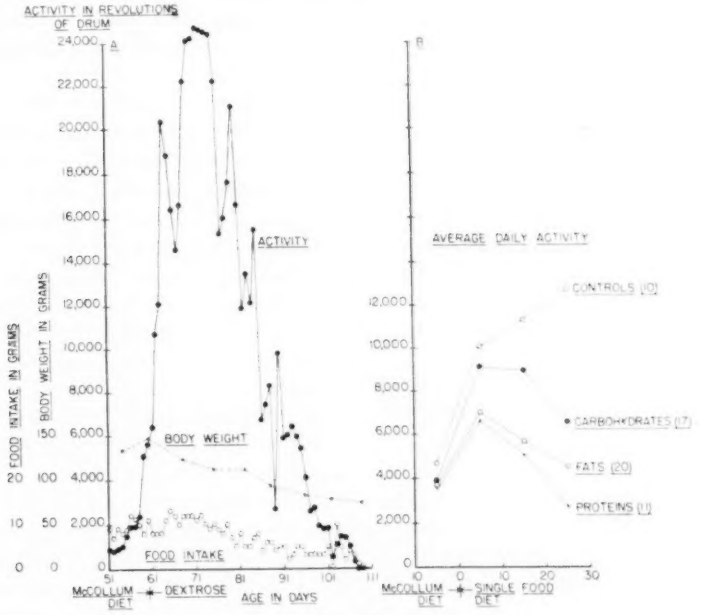

Fig. 4

During the second 10-day period the rats on the carbohydrates did not become more active; but they maintained their previous level of activity, while those on fats and proteins became much less active. During the third 10-day period all of the experimental rats became less active. At this time the rats on carbohydrates were still about 3 times as active as the rats on proteins. Even then the rats on the carbohydrates were about half as active as the control rats on the McCollum diet.

Two of the rats on dextrose were the most active of any of the 116 rats. Figure 4A gives the record of one of these animals. During the first 20 days this rat on dextrose alone had a daily average of more than 22 miles,

definitely surpassing the normal averages. Among the rats on the carbohydrates, the rats on sucrose and maltose were next most active. Of the rats on fats, those on butter, peanut oil and olive oil were most active, while those on Crisco and perilla oil were least active. Of the rats on proteins, those on casein (Labco) were most active, while those on hemoglobin were least active.

Water intake. Carbohydrates. The rats on carbohydrates, excepting galactose, manifested a markedly diminished thirst. Figure 5A includes the average daily water intake curves of the groups on casein (McCollum), dextrose and olive oil, and of 27 female controls on the McCollum diet. The average water intake of the rats on dextrose decreased from 26 cc. per day during the last 10 days on the McCollum diet to 7 cc. during the following 30- to 40-day period.

The four rats on galactose gave entirely different results. These animals began drinking large amounts of water within a day or two after the single substance was offered. Figure 5B shows the individual water intake curves of the four animals on galactose. The water intake of one rat (no. 1) increased from an average daily intake of 25 cc. during the last 10 days on the McCollum diet to 71 cc. during the remainder of its survival period on galactose and on one day reached a peak of 125 cc.

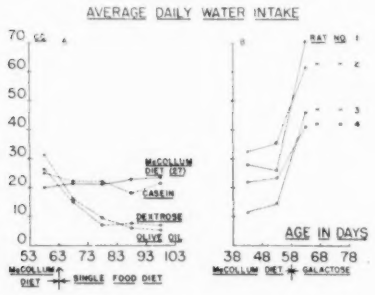

Fig. 5

Fats. Rats on pure fat diets also had a reduced thirst. Figure 5A includes the average daily water intake curve for the rats on olive oil, which is typical for the other fats. The water intake decreased from a level of 31 cc. per day during the last 10 days on the McCollum diet to a level below 10 cc. 20 days later, where it remained until the death of the animals. The 3 rats on glycerine drank large amounts of water during the few days that they survived. Their intakes reached peaks of 75, 117 and 123 cc. respectively.

Proteins. In contrast, the rats on most of the proteins continued to drink about the same amount of water as on the McCollum diet. The rats on the casein digests, 92-Z and 89-7, and on desiccated blood fibrin drank even more water for the first 20 days. Figure 5A shows that the average daily water intake curve for the group offered casein (McCollum) decreased only very slightly and closely approximated the control average throughout the experiment.

Estrus cycles. In most of the rats the vaginal smears failed to show

cornified cells at any time after the start of the single food diet. In some of the rats, especially those on fats and carbohydrates, the smears showed one and occasionally two full 4- to 5-day cycles of cornified cells for the first 8 to 10 days and thereafter showed only a typical diestrus picture. In a few rats (3 on carbohydrates and 7 on proteins) cornified cells reappeared in the smears sporadically after the start of the diet. In these rats the smears showed constant cornification for short periods of 3 to 10 days at irregular intervals until the rats died. The appearance of the constant cornification of the smears might indicate the presence of a vitamin A deficiency. However, we did not detect any other symptoms of vitamin A deficiency, such as keratitis, in any of these animals.

Noteworthy is the fact that in some of the animals the 4- to 5-day estrus cycles manifested themselves in the running activity long after they had disappeared from the vaginal smears. Some of the rats on dextrose and maltose showed 5 regular 4- to 5-day activity cycles. The fact that the activity cycles were more apt to persist in the rats on carbohydrates may be associated with the higher activity level of the carbohydrate rats. In one of the rats on maltose the 4- to 5-day cycle manifested itself more clearly in the water intake than in the vaginal smears or activity. This rat showed 7 definite cycles during which the water intake decreased and increased at 4- to 5-day intervals.

The persistence of the activity cycles after the disappearance of the vaginal smear cycles must indicate that the ovaries were still functioning. This situation is similar to that found in vitamin A deficiency, when the vaginal smears show constant cornification, but the activity still shows the regular 4- to 5-day cycles. The persistence of the 4- to 5-day activity cycles in vitamin A deficiency indicates that the ovaries are still functioning.

The disappearance of the cornified cells of the vaginal smears of the rats on the single food diets must be due primarily to a deficiency of the vitamin B complex. Almost identical records are obtained when the rats have a full diet except for the vitamin B complex.

Discussion. The results of these experiments have shown that rats may live a surprisingly long time, 50 days and more, on diets which contain only one of the three main foodstuffs, either a carbohydrate, a fat or a protein. Even more remarkable is the fact that they are able to survive so long when, as was true particularly of the rats on carbohydrates, their diets contained neither minerals nor vitamins. It seems especially noteworthy that the animals did not develop any of the classical signs of either mineral or vitamin deficiencies. Rats on regular diets, but deficient in vitamin A, develop symptoms of vitamin A deficiency in 20 to 30 days. None of these single food animals showed keratitis; a few showed irregular periods of cornified smears; none showed any outward signs of vitamin D deficiency. Chemical studies of bones were not made, but many of the

rats were still turning the drum several thousand revolutions per day after they had been on a single food diet for over 40 days. Clear cut vitamin B deficiency symptoms did not appear either—skin and hair on the snouts and around the eyes or on the paws did not show the changes found with a marked deficiency of any one of the vitamin B components. These results agree with observations of McCollum et al. (1922) which showed that complete starvation relieved vitamin D deficiency symptoms.

From these results the possibility suggests itself that in the usual experiments in which synthetic or natural foods were used the appearance of pathological symptoms depended more on harmful effects which resulted from eating too much of certain substances rather than too little.

These experiments have shown, further, that rats manifest widely different appetities for different single foods. The discussion of the significance of these differences must take into account the following considerations:

The daily intake of a single food will depend on: 1, *taste*: the animals may eat more dextrose than lactose simply because they prefer the taste of the dextrose; 2, *digestibility*: for example, a limited amount of a lactose splitting enzyme might be responsible for the small amounts of this sugar taken by these rats; 3, *absorption*: both the ease and the rate of absorption of the products of digestion must have an influence on the amount of a given food voluntarily ingested; 4, *satisfaction*: the animals may eat more dextrose because its consumption is followed by a relief from hunger or stop eating cod liver oil because of some sort of internal distress; 5, *excretion*: for example, it is possible that the animals eat less of the proteins just because they have metabolic end products which must be excreted through the kidneys, while the carbon dioxide and water produced from the carbohydrates and fats can be completely eliminated by the lungs and skin.

In addition, the survival time would depend on: 1, the *amount* of food absorbed; 2, the *utilization* of the absorbed food, which means essentially its ability to maintain a constant internal environment in the animal, as evidenced by a constancy of the blood levels of the products of absorption and of excretion within normal limits; 3, the *completeness*, or the nutritional value, of the absorbed food; 4, *toxic effects*.

Obviously it is difficult or impossible to evaluate in animals some of the factors listed above. This is particularly true of taste and satisfaction, which are, per se, subjective considerations. However, there are enough objective factors to justify an attempt at the interpretation of the data.

When the carbohydrates are considered on this basis, one is not surprised to find that the animals survived longest and were most active on dextrose, since this is the form in which all carbohydrate is utilized in the body. The rats on maltose did not get along as well; the small difference can presumably be attributed mainly to the digestion factor, since maltose is hydrolyzed to two molecules of dextrose. The animals on starch did not

survive as long as and were less active than the two preceding groups. This was all the more unexpected since the food consumption during the initial 10 days on the diet was greatest in the starch group. Since starch also produces only dextrose on hydrolysis, the most likely explanation seems to be a failure of starch digestion, either through an inability to produce enough of the starch splitting enzymes or, perhaps, through a limitation of the neutral salts necessary to the activity of the pancreatic amylase (Sherman, Caldwell and Adams, 1930). The animals on sucrose, which yields dextrose and levulose on hydrolysis, did not do as well as those on maltose, while those on levulose alone, on lactose (yields dextrose and galactose), and on galactose alone were obviously inferior in nutrition. The poor results with galactose are in accordance with the fact that mammals, in general (and including man), have a very low galactose tolerance and that the excess of this sugar is excreted through the kidneys, thus accounting for the polyuria and compensatory polydipsia attending the feeding of this carbohydrate. Furthermore, the work of Schantz and his collaborators (1938) has indicated that galactose cannot be oxidized in the animal body in the absence of saturated fatty acids containing 12 or more even numbered carbon atoms.

With the fats the conditions are more complicated since they were not all obtainable in a chemically pure state. In particular, the amounts of fat soluble vitamins present may have had a significant effect in some cases. In first place of interest is the fact that the daily intake of the fats, as measured in grams, was approximately half as high as that of the carbohydrates; yet when calculated in terms of calories, the intakes of these two foodstuffs were almost identical. Bulk of the food clearly played no part at all. It may be assumed that the rats took just as much of the fats as they were able to absorb. The marked differences in survival times would depend on how well the fats could be utilized and the nutritional values of the food. That butter and olive oil showed the longest survival times agrees with our knowledge of their utilization and of their vitamin contents. With survival times as short as these it seems extremely unlikely that a deficiency of any essential fatty acid (as, for example, linoleic acid) can have played a significant part in causing death. The shorter survival time of the rats on cod liver oil may have been due not to the inability to utilize the fat but to the toxic action of the excessive amounts of vitamins A and D. The shorter survival time of the rats on wheat germ oil, peanut oil, and perilla oil may be accounted for in part by toxic effects and in part by possible deficiencies of essential fatty acids. Survival experiments with the individual fatty acids offered singly or in various combinations should throw further light on this problem. Attention may be called to the fact that on glycerine the rats lived longer than on no food at all. That the appetite for fats may be controlled ex-

perimentally through a wide range has been shown by recent self-selection experiments; for example, removal of the pancreas greatly increased the fat intake (Richter and Schmidt, 1940), and rats kept on a vitamin B deficient diet also showed a marked craving for fat (Richter and Hawkes, 1940), presumably due in both cases to the decreased efficiency of carbohydrate utilization.

The determination of significance of differences in food intakes and survival times of the rats on the proteins also offers more difficulty than that encountered in the study of the carbohydrates. Although the caseins were freed from the fat and water soluble vitamins, they still contained high amounts of phosphorus and other minerals; the hemoglobin and desiccated blood fibrin contained iron, other minerals, and some vitamins. In general, the results were much less consistent than with the fats and carbohydrates. Some rats on casein lived only 15 to 20 days; several survived 45 days or longer.

Measured in grams, the rats ate less of the proteins than of the carbohydrates and fats. Also measured in calories, the protein intake fell below the level of the other two foodstuffs. Furthermore, the protein intake tended to remain essentially the same throughout the experimental period, independent of the changes in body weight.

Since the intake of the different proteins did not vary much, it may be assumed that they were fairly equally absorbed; and the large differences in survival time may be accounted for mainly by the difference in nutritional value, the amino acid content. The caseins are known to contain all of the essential amino acids, while desiccated blood fibrin lacks at least isoleucine, hemoglobin is very low in cystine, and lactalbumin lacks methionine and probably other essentials. Zein, which the rats ate in very small amounts, lacks several essential amino acids. The long survival time of some of the rats on the enzyme casein digest may be accounted for by the fact that this digest contained all of the essential amino acids. Seventy-five per cent of the nitrogen in this preparation was the result of casein hydrolysis, while the remaining 25 per cent was from the pancreas used for digestion. The shorter survival time of the rats on the acid digest, 89-7, may be explained, at least in part, by the destruction of tryptophane and other amino acids. The fact that the rats ate only minimal quantities of zein indicates that taste must play a very important rôle in the determination of food intake.

These experiments, in which the rats had access to only one food, make it possible to establish the relative dependence of spontaneous activity on each of these three foods. The results show that on the carbohydrates the rats were most active, less active on the fats, and still less active on the proteins.

Further, it appears that the level of water intake depends on the type of food the animals are offered. Fats and carbohydrates, with the excep-

tion of galactose and glycerine, definitely reduce the water intake, whereas protein maintains the water intake at approximately the normal level. As has been suggested, this may be because the nitrogen of protein must be excreted through the kidneys. The high water intake of rats on galactose may be explained similarly, since the experiments of Schantz et al. (1938) and Cori (1925) show that the galactose is excreted in the urine. The high intake of the rats on glycerine may be explained by the marked dehydration produced by the glycerine.

In 1816 Magendie performed the first single food experiments with purified (or nearly purified) substances. He was interested in determining the source of the nitrogen of the tissues. He kept dogs on water and either sugar, olive oil or butter. On water alone they lived 10 to 12 days; on olive oil, 36 days; on butter, 36 days (but the dogs had meat on the 32nd day). He did not give the age of the animals. He found that olive oil, sugar or butter kept the animals alive approximately the same length of time. Phillips in 1924 used the single food technique to study the nutritional value of the various carbohydrates for bees. He found that honey bees would eat dextrose, maltose, sucrose, levulose, trehalose and melezitose and, when offered any one of them, definitely outlive the experimental controls kept on no food. They refused galactose, lactose, raffinose, xylose and the more complex polysaccharides—starch, dextrins, inulin, etc. He concluded that the sugars which the bees would eat were utilized and the others which they refused were non-utilizable. von Frisch repeated Phillips' experiments and obtained very similar results (1927, 1928, 1930). The refusal of honey bees and rats to eat either lactose or galactose, while they avidly eat dextrose and sucrose, is especially noteworthy. The results obtained in the present study on rats stand in close agreement with those obtained by Phillips on bees.

SUMMARY

1. Rats were kept in separate cages on a standard food mixture until they reached an average age of 64 days. Then their diet was restricted to water and a single purified (or nearly purified) foodstuff – a carbohydrate, a fat or a protein. The survival time, activity, etc., on the single foods was taken as a measure of their nutritional value.

2. With this single food choice method a survey was made of 7 carbohydrates, 9 fats and 10 proteins.

3. Rats showed the greatest appetite in the case of the carbohydrates for starch, dextrose, and sucrose; in the case of the fats, for butter, olive oil and lard; in the case of the proteins, for desiccated blood fibrin and casein. They showed the smallest appetite in the case of the carbohydrates for lactose and galactose; in the case of the fats, for perilla oil and glycerine; in the case of the proteins, for zein, lactalbumin and gelatin.

4. They live longest on the carbohydrate, dextrose (57 days). One of

the fats (butter) kept them alive 53 days; and the protein, casein (enzyme digest), gave an average survival time of 47 days. For the carbohydrates and fats the food intake bore a direct relationship to survival time; for the proteins this relationship did not hold.

5. On the proteins the rats lost weight during the first 10 days somewhat more rapidly than on the carbohydrates and fats. Later on the rats lost weight at about the same rate on each of the three foodstuffs.

6. The rats on the carbohydrates were the most active; those on the fats, less active; those on the proteins, the least active.

7. Most of the rats on the single foods showed diestrus vaginal smears almost immediately after starting on the single food choice diets.

8. Rats on fats or carbohydrates, excepting galactose and glycerine, drank comparatively small amounts of water; rats on proteins drank normal amounts. The rats on galactose and glycerine drank very large amounts.

9. The results indicate that in usual experiments in which mixtures of synthetic or natural foods were used the appearance of pathological symptoms depended more on the eating of too much of certain substances, rather than too little.

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THE RESPONSE TO INTRAVENOUSLY INJECTED DEXTROSE IN RATS ON NORMAL AND B_1 DEFICIENT DIETS

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The tolerance to orally administered dextrose has been found to be decreased in vitamin B_1 deficiency in animals (1, 2) and in humans (3). The reason for this diminished tolerance has not been adequately explained. One of the possible contributing factors which has been suggested is the change in motility of the intestinal musculature which occurs in vitamin B_1 deficient as well as in starved animals (4). In order to eliminate this factor of absorption, dextrose has been injected intravenously in normal rats, and in rats fed a vitamin B_1 deficient diet.

METHOD. Male rats weighing between 135 and 145 grams were used. The normal diet was made up as follows: casein, 15 per cent; yeast, 10 per cent; Crisco, 20 per cent; cod liver oil, 2 per cent; sugar (cane), 50.5 per cent; salt, 2.5 per cent. The diet was made B_1 deficient by treating the yeast with sulphur dioxide, according to the method suggested by Kline (5).

Four groups of rats were used: group 1, normal diet, ad libitum; group 2, vitamin B_1 deficient diet, ad libitum; group 3, deficient diet, restricted to a weighed amount of food each day; group 4, normal diet, restricted to the same amount as given to group 3.

The amount of food eaten by each rat was calculated by weighing the food left at the end of each day and subtracting it from the total given.

Food was withheld for 24 hours before each test. Water was allowed ad libitum.

The tail veins were used for the intravenous injection. A small amount of xylol was applied to the skin of the tail in order to make the veins prominent. A 26 gauge needle and tuberculin syringe were used; 0.5 gram of 20 per cent dextrose in normal saline per kilogram of body weight was administered intravenously. Fasting, 5 minute, 15 minute, 30 minute and 60 minute blood specimens were obtained in the following manner:

The tail was immersed in warm water (45–50°C.), wiped dry and then clipped at its end. The first drop was discarded and the blood was then gently expressed on to a clean glass slide. One-tenth cubic centimeter of blood was then drawn up into a micro-pipette. Bleeding was controlled by the use of collodion and cotton.

TABLE 1

RAT NUMBER	AGE	WEIGHT	INTRAVENOUS GLUCOSE TOLERANCE (BLOOD SUGAR MGM. PER CENT)					DAYS ON DIET	PER CENT WEIGHT LOSS	SYMPTOMS*
			Fasting	(Minutes after injection)						
				5	15	30	60			
			B_1 deficient diet ad libitum							
	days	*grams*								
1	63	165	100	200	143	116	100	25	14	P+ S+
	71	162	110	181	148	121	110	33	15	H+ P++ S+
	79	146	85	165	133	113	105	41	24	H+ P++ S++
	79	162	77	143	113	98	110	30	19	P+ S+
2	86	142	101	179	113	92	87	37	26	H+ P+ S+
	93	122	125	240	190	152	125	44	39	H+ P++ S+
3	46	172	60	152	92	63	80	13	3	0
	53	170	63	154	125	105	85	20	0	P+ S+
	60	151	90	188	163	133	110	27	20	H+ P++ S+
	54	202	72	165	147	110	90	16	0	0
4	69	165	84	177	165	116	94	31	18	P± S
	76	150		275	187	143	104	38	26	H+ P++ S+
	56	160	66	160	133	100	80	18	9	P±
	69	126	82	250	200	180	115	31	28	H+ P++ S++
5	55	162	82	202	153	142	100	11	0	0
	79	128	108	330	240	165	133	35	26	H++ P++ S++
			B_1 deficient diet (restricted—see text)							
6	55	166	67	194	138	112	97	11	0	0
	79	160	95	220	200	160	122	35	7	H+ P+ S+
7	76	142	83	184	134	118	105	35		H+ P+ S+
8	63	153	94	222	147	139	108	22		0
9	72	140	66	179	175	137	117	30		P+ S+
			Normal diet ad libitum							
10	60		102	181	146	121	91			
	67		66	174	160	123	100			
11	57	164	73	190	153	145	97			
	79	228	86	200	154	118	90			
			Normal diet (restricted—see text)							
12	59	159	75	167	122	110	94	17		
	73	160	78	200	140	112	100	31		
13	57	160	63	180	145	120	85	13		
	84	168.5	94	205	167	122	102	40		
14	76	154	78	167	123	110	95	35		
15	72	162	80	157	121	109	91	30		
16	57	156	70	175	133	111	95	16		

* P, polyneuritis; S, spasticity; H, hump.

Blood sugar was determined by the micro method of Folin (6).

RESULTS. Rats which had been fed on the normal diet (ad libitum or restricted) had a fairly uniform response to intravenously administered dextrose (table 1). These findings are similar to those obtained in children (7). The animals which had been given the B_1 deficient diet (ad libitum or restricted) usually exhibited a decrease in intravenous dextrose tolerance, and an increase in the fasting blood sugar, as the deficiency progressed (table 1). The decrease in tolerance is apparently independent of food intake.

Two rats weighing 190 grams each who had been on normal diets, were starved for 72 hours. Water was allowed ad libitum. The intravenous dextrose tolerance failed to show a decrease in tolerance.

TABLE 2

RAT NUMBER	WEIGHT	FLUID INJECTED	INTRAVENOUS GLUCOSE TOLERANCE (BLOOD SUGAR MGM. PER CENT)				
			Fasting	Minutes after injection			
				5	15	30	60
Normal diet							
	grams						
17	182	Normal saline 0.4 cc.	85	91	92	80	100
18	200	Normal saline 0.5 cc.	102	117	117	95	90
19	206	None	89	95	95		96
B_1 deficient diet							
20	180	Normal saline 0.4 cc.	95	110	105	102	107
21	143	Normal saline 0.35 cc.	80	100	95	90	85
22	141	None	73	94	98	81	90

Normal saline was injected intravenously into four rats, two of which had been on normal diets and the other two on vitamin B_1 deficient diets. Blood sugars determined at various intervals showed no significant change from the fasting level (table 2).

The tail veins of a normal rat, and one who was vitamin B deficient, were punctured, but no fluid given. Blood sugars showed no significant variation during an hour period.

SUMMARY. 1. A method for determining the tolerance of the rat to intravenously injected glucose has been described.

2. Rats which had been on a normal diet have a fairly constant response to dextrose administered intravenously.

3. Rats, which had been fed a vitamin B_1 deficient diet show a progressive decrease in tolerance, which is apparently independent of food intake.

CONCLUSION

The decrease in oral glucose tolerance which occurs in rats fed a vitamin B_1 deficient diet, is not due to changes in absorption from the intestine, since this decrease also occurs when dextrose is administered intravenously.

I wish to thank Dr. Elizabeth M. Knott of the Department of Pediatrics for her aid in this work.

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RIBOFLAVIN DEFICIENCY IN THE PIG

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Studies of nutritional deficiency in the pig have not established that riboflavin is essential to life in this animal. Although the experiments by Chick and her co-workers (1) revealed the need of the pig for other components of the vitamin B_2 complex, they did not show whether riboflavin is an essential dietary constituent for the pig. Hughes (2) observed "that pigs fed a diet deficient in riboflavin gained very slowly, defecated frequently semi-liquid fecal material, became crippled, and walked with difficulty." In a more recent report (3) Hughes estimated from growth curves that the minimum daily requirement of riboflavin for the young growing pig lies between 1 and 3 mgm. per 100 pounds of pig. Wintrobe (4) likewise observed that the addition of riboflavin to a diet deficient in the B complex accelerated the rate of growth.

It seemed possible that with the production of chronic riboflavin deficiency in a susceptible animal, there might arise pathologic changes which would not have had time to develop in acute riboflavin deficiency. With this in mind, 6 pigs were placed on a dietary regimen only partially deficient in riboflavin. Two of the 6 pigs, serving as controls, received daily supplements of synthetic riboflavin in addition to the basal diet. In the present report the two pigs fed the basal diet plus daily supplements of riboflavin will be designated "control pigs."

The clinical differentiation between those pigs fed a diet deficient in riboflavin and the control pigs was striking in several respects. The animals fed the riboflavin deficient diet developed similar changes in the eyes, coat and gait, and they ultimately experienced a characteristic collapse. Since the control animals did not develop these changes, they were attributed to the lack of riboflavin. In both groups, however, anemia appeared after varying intervals of time. On this account it is felt that the anemia was a manifestation of a second deficiency state.

Dietary. The diet was patterned closely after diet no. 330 employed by Sebrell (5) for the production of riboflavin deficiency in dogs. In preparation our diet differed chiefly in that the rice polishings were not ex-

tracted with ether and the casein was not leached. Chemical analysis of these substances showed the presence of small amounts of riboflavin. All animals received identical, weighed diets during the period of observation. The caloric intake was restricted for two reasons: first, in order to assure isocaloric feeding of both deficient and control animals; and second, in order to prevent growth to an ungainly size, which was not feasible for practical reasons. The maximal weight attained (by the controls) was 25 kilos over a period of 6 months.

The control animals received synthetic riboflavin[1] 2.5 mgm. daily, which provided at least 100 gamma per kilo body weight daily.

All of the animals ate ravenously until within 24 to 48 hours of death. None developed signs of polyneuritis. None developed the signs of pellagra that have been described in pigs (1).

In the course of the present studies, it became evident that rice polishings in the amounts fed did not satisfy the pigs' needs for an additional factor or factors contained in the "B complex". This additional deficiency was manifested by anemia and spastic paralysis.

Observations. The studies were made on 6 Cheshire white pigs, litter mates, of which 4 were females and 2 were males. The pigs were reared on whole cow's milk until seven weeks of age. Thereafter they were fed the diet outlined in table 1. The pigs were housed in separate cages throughout the course of the experiment. At no time did they develop intercurrent infections. The two male pigs (one of which served as a control) were castrated at the age of 5 months. Blood for analysis was obtained at monthly intervals by cutting the tails.

Growth. The pigs consumed their diets until within a day or two of death. The stools were of normal bulk and consistency. There were no periods of vomiting or of diarrhea until the animals were moribund, when the stools usually became semi-liquid. Although the food intake of both deficient and control animals was identical, the control animals grew more rapidly and attained a greater size. This implies that riboflavin accelerated the rate of growth, independently of the food intake. Hughes (6) likewise has noted that the addition of whey adsorbate to the diet of pigs allowed more "efficient food utilization". After reaching a maximal weight the values declined, and later rose again. The cause of this fluctuation in the control animals was not ascertained. Since the decline in weight coincided in time with the onset of anemia in both the deficient and control animals, it is believed that the decline in weight probably marked the clinical onset of a secondary deficiency state. These changes are illustrated by figure 1, which shows the growth curves and hemoglobin values for a control pig and for a pig (no. 4) on the riboflavin deficient (basal) diet.

[1] Furnished by Merck and Company, Rahway, New Jersey.

Body temperature. During the first 3 months of observation the rectal temperatures were 102.6° to 103.4°F. Thereafter, a decline of body temperatures to 96° to 100°F. was noted. The control animals also showed this tendency to a lesser degree. Hughes (6) has recorded similar, but less marked reduction in body temperature of pigs fed diets deficient in various components of the "B complex", which, however, were not sharply defined. It is likely that undernutrition in general, rather than the specific want of riboflavin was responsible for the change. With the

TABLE 1

Composition of riboflavin deficient diet (per animal)

	GRAMS	PROT.	CHO	FAT
1. Rice polishings	80.	11.4	54	9.6
2. Cotton seed oil	22.			22.
3. Corn starch	131.		118.	
4. Casein	41.	35.6		
5. Cod liver oil	8.2			8.2
6. Salt mixture	12.0			
Total		47.0	172.	39.8

Total calories, 1236.

1. Rice polishings untreated. Chemical analysis revealed 5 gamma/gram of riboflavin.[2]

2 and 3. Commercial grades.

4. Casein Co. America. Grade 20. Chemical analysis revealed 2 gamma/gram of riboflavin.[2]

5. Cod liver oil—contained 1800 i.u. vitamin A and 260 i.u. vitamin D per gram.

6. Hawk-Oser Salt Mixture (Science **74:** 369, 1931). To this salt mix were added nicotinic acid 5.5 mgm. and ascorbic acid 50 mgm.

Water was added to the mixture of rice polishings, casein and cotton seed oil until a thick gruel was formed. This mixture was cooked in a double boiler for 1½ hours. The other ingredients were then added in and the final mixture served.

Freshly prepared solutions of synthetic riboflavin (Merck) were added to the diets of the control pigs just prior to serving.

onset of collapse, there was a further sharp fall in body temperature to values below 94°F. (the lowest reading registered on the thermometers).

Changes in the hair and skin and hoofs. Throughout the course of the experiment (12 mos.) the hair of the control animals was white, clean, and lustrous. The skin was free from ulcers. The hoofs were smooth and shiny.

After 3 to 4 months the skin of the animals fed the basal diet alone became scaly and ulcerated, especially on the snout and about the hoofs.

[2] Analyses were made by Dr. Joseph W. Ferrebee, by the method described in the J. Clin. Investigation **19:** 251, 1940.

The skin of the snout, in 3 animals, was thickened and cornified. The eyelids appeared swollen and the palpebral fissures narrowed. The horn of the hoofs became deeply pitted and ridged. The hair became grey, rough, and thin, especially about the eyes, haunches, and back. These findings in the pig bear a close resemblance to the changes observed to occur in rats on riboflavin deficient diets (7).

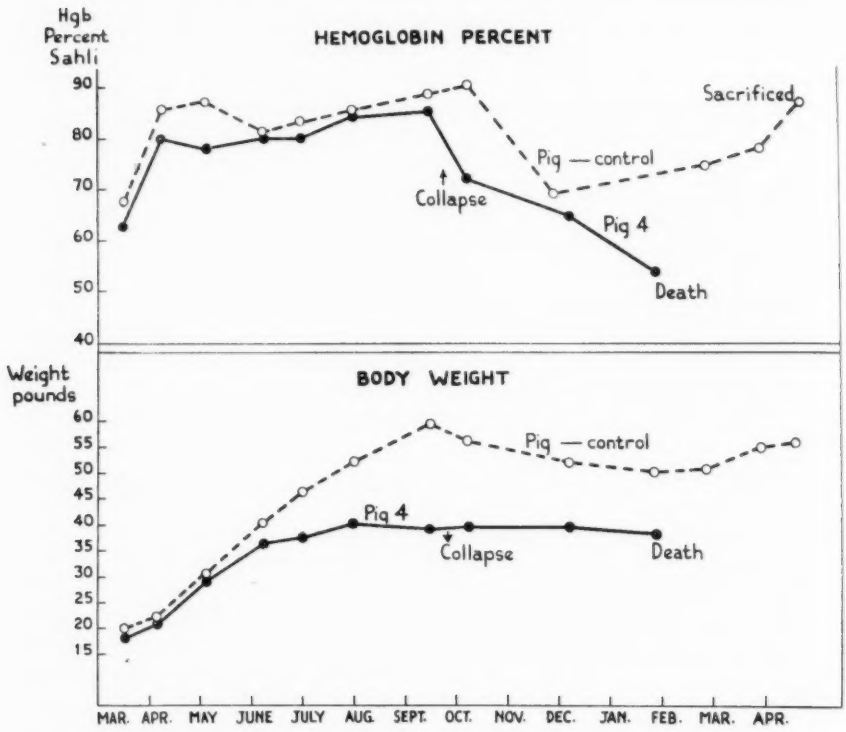


Fig. 1. Weight and hemoglobin curves of control pig and of pig on riboflavin deficient diet.

Eye changes. After 5 months the corneas of 3 pigs fed the basal diet became cloudy. An ophthalmological examination by Dr. Maynard Wheeler at this time revealed that the corneal surface was smooth and glistening, free of scars or ulcerations. The light reflex was normal. There was no apparent vascularity or inflammation of the cornea or conjunctiva. However, slit-lamp examination was not made. The lens and

fundus appeared to be normal. The cloudiness, therefore, was attributed to changes in the deeper stroma of the cornea. The cloudiness increased until, in extreme cases, the entire cornea became an opaque white, so that the underlying pigment of the iris was hidden from view. The external corneal surface retained its lustre.

In two animals (pigs 3 and 4) the cloudiness cleared partially after the administration of riboflavin, so that in 2 and 10 days respectively the peripheral zones again revealed the underlying markings of the iris. In these cases there now remained a dense, central opacity which resembled a cataract.

Gait. No neurological changes were observed in these animals until the onset of collapse. Tendon reflexes and sensation (response to pin prick) were intact. About the third month, however, the animals fed the deficient diet were seen to be "flat footed." The hoof digits became spread widely apart, so that the weight of the animal was borne by the heel, or false hoof. Since there was no apparent neurological involvement at this time, this change was attributed to muscle weakness or tendon relaxation.

Collapse syndrome and response to riboflavin. The collapse syndrome was similar to that observed in dogs (8, 9, 10). There was considerable variation in the time of onset. Collapse occurred in the 4 pigs on the basal diet at 3, 6, 7 and 10 months, respectively, after the experimental feeding was begun. The pigs suddenly became listless, and they refused to eat and to stand. The body and extremities were cold and cyanotic. The body temperatures were below 94°F. and the pulse and respirations were slow and irregular. Electrocardiograms showed minor changes of ventricular complexes. When the collapse was profound, respirations became asthmatic, with a wheezing expiration audible with the stethoscope. No blood flowed from the cut tail. Reflexes were absent. Pin prick over the legs, body, and snout elicited no response. One animal (no. 1) died 24 hours after the onset of this syndrome.

Riboflavin in isotonic saline (1 mgm. per cc.) was administered to three animals in collapse. One animal (no. 2) received 20 mgm. of riboflavin parenterally 24 hours after the onset of collapse, but it died 5 hours later. The two other animals (nos. 3 and 4) were revived by the parenteral administration of riboflavin. Pig 3 received 100 mgm. intravenously 3 hours after the onset of collapse. Pig 4 received 200 mgm. intravenously and intraperitoneally 2 hours after the onset of collapse. The response to these injections was dramatic. Suddenly revived from collapse, the animals grunted, stood up, walked, and ate their food. The animals were unsteady on their hind quarters for several days, following which ataxia disappeared. The basal diets were continued. Without further injections of riboflavin one pig (no. 3) lived 2 weeks and the other (no. 4)

lived 4 months thereafter. At the end of this time they died of another syndrome, which was characterized by spastic paralysis of the hind legs and rapidly progressive anemia.

Response to glucose. Although hypoglycemia was a feature of the collapse syndrome, certain data suggest that this syndrome was not the direct result of hypoglycemia. In one animal (no. 1) the blood glucose was 50 mgm. per cent at the onset of collapse. Twenty-four hours later, when the blood glucose had fallen to 20 mgm. per cent, the pig was given 100 cc. of 5 per cent glucose in isotonic saline intraperitoneally. Following the injection the pig stood up and walked for 15 minutes. One hour later it suddenly died. In another animal (no. 3) the blood glucose fell from a previous value of 62 to 23 mgm. per cent at the onset of collapse. The pig was given 50 cc. of 50 per cent glucose intravenously. The animal was roused for 10 minutes, but it was unable to stand. It then lapsed into deep stupor. One hour after the glucose injection the blood glucose was 625 mgm. per cent; two hours after the injection it was 500 mgm. per cent. The pig appeared to be moribund. When a further half-hour had elapsed riboflavin was administered, following which abrupt recovery took place. Three hours later, when the pig appeared to be vigorous and well, the blood glucose was 96 mgm. per cent.

It is also of interest that 2 weeks following this recovery in pig 3, the blood sugar fell to low values (16 to 30 mgm. per cent) for 3 days without accompanying collapse.

Anemia and spastic paralysis. Two animals, pigs 3 and 4, recovered from the initial collapse after the parenteral injection of riboflavin. In the following 2 weeks pig 3 developed a rapidly progressive anemia and spastic paralysis from which it died. Pig 4 died after 4 months, from an entirely similar syndrome. In neither case was this latter syndrome accompanied by collapse.

Moreover, after 10 months, the two control pigs developed a moderate weight loss and transient anemia, followed by spontaneous recovery.

On this account it seems possible that the terminal anemia and spastic paralysis of pigs 3 and 4 (cf. fig. 1) were due to a partial lack of another factor or factors in the "B complex". The spastic paralysis resembles the changes described in dogs maintained on B-deficient diets (11). The anemia resembles that attributed to the lack of "eluate fraction" by Chick (1) and her co-workers.

Other blood determinations. The first blood determinations made when the pigs were 2 months old revealed hypochromic anemia. This probably was related to the previous milk diet, since a prompt rise in hemoglobin followed the change to a synthetic diet. The red blood cell counts and hemoglobin values thereafter were normal, until the syndrome of spastic paralysis and anemia supervened, as described above.

The white blood cell counts fluctuated widely. Their usual range was between 12000 and 25000 cells per cu. mm. There was no apparent cause for this fluctuation.

The serum proteins were essentially unchanged throughout the period of study. In general, the serum albumin level for the pigs was between 4.0 and 5.0 grams per 100 cc., and the serum globulin level, between 1.8 and 3.5 grams per 100 cc. The nonprotein nitrogen varied from 22 to 41, the average value being 27 mgm. per 100 cc.

Necropsy. *Gross* and *microscopic* examination of the 2 control pigs revealed no pathologic changes.

Gross findings of the 4 animals on the basal diet were very similar: there was marked diminution of subcutaneous and mesenteric fat. Skin over the lower legs was desquamated and ulcerated. Mucous membranes were pale. Tongue papillae were normal. The cornea of 3 pigs showed differing degrees of opacity. There were no gross changes in the thoracic or abdominal viscera, or in the brain and spinal cord. Diffuse petechial hemorrhages were seen in 2 pigs (nos. 1 and 2).

The chief *microscopic* findings of the 4 pigs on the riboflavin deficient diet were as follows: In 4 pigs the cornea showed changes in the basal cells of the surface epithelium. These cells were swollen, cuboidal, and irregular, and they were covered by a layer of one or two flattened cells and by cornified epithelium. There was no vascularization of the cornea. Figure 2a-b illustrates the above changes.

In 3 pigs the cortex of the adrenals showed recent hemorrhages, together with loss of vacuolization of the cells of the glomerular layer, and an increase in the interstitial stroma.

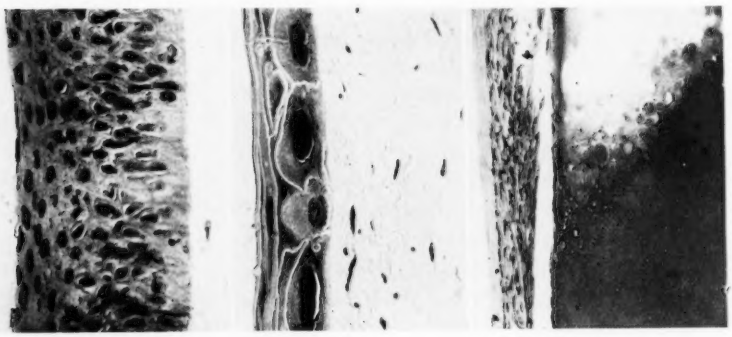
In 2 pigs the kidneys showed vacuolization in the proximal convoluted tubules, due to the presence of neutral fats and of anisotropic crystals. In one pig the lens of the eye showed proliferation of the subcapsular epithelium, similar to that described by O'Brien (12) in young rats on G deficient diets (cf. fig. 2-c). In one pig the liver showed rare areas of focal necrosis.

No significant changes were found in the heart, lungs, stomach, spleen, pancreas, brain, spinal cord, sciatic nerve, pituitary, or thyroid glands.

Discussion. From the preceding data it is apparent that riboflavin is essential for the pig. Since corneal changes occurred in the animals fed a riboflavin deficient diet but not in the two control litter mates, this eye lesion appears to be directly or indirectly the result of riboflavin deficiency. Although possibly somewhat different in nature, corneal lesions due to riboflavin deficiency also have been observed in the rat (7, 13), and more recently in man (14).

In contrast to the control animals, the pigs on the basal diet alone exhibited changes in the hair, skin and hoofs.

The mechanism of the collapse syndrome is not clear. The association of collapse with hypoglycemia suggested the possibility of hypoglycemic shock or of adrenal failure. However, there are two observations suggesting that the hypoglycemia is an associated phenomenon rather than an immediate cause of the collapse: Two pigs, given glucose infusions during their collapse, derived transitory stimulation. One pig rose to his feet, moved about for 15 minutes, and then suddenly died. The other pig likewise became somewhat roused for 10 minutes. It then relapsed into profound shock in spite of hyperglycemia. The syndrome, therefore, is unlike hypoglycemic shock, in which a more lasting benefit is derived from



a b c

Fig. 2a. Control pig. Normal corneal epithelium (magnified × 300).

b. Corneal epithelium of riboflavin deficient pig 4 (magnified × 300). The epithelial layer is about one-third the usual thickness. The parallel arrangement of cells is lost. Basal cells are swollen, and their hypertrophied nuclei often contain large vacuoles. The superficial cells are cornified.

c. Optic lens pig (no. 4). Proliferation of the subcapsular epithelium with infiltration of the lens substance. There is degeneration of the lens fibers with vacuolization and formation of Morgagnian bodies (magnified × 70).

the administration of glucose. It is possible that riboflavin deficiency interferes with the effective utilization of glucose.

In one instance the blood sodium was low (129.8 ml. equiv.) at the time of collapse. In two instances the blood sodium did not differ from that of a control animal. In one instance the blood sodium before and after recovery from collapse showed no significant changes. It is of interest, and of possible significance, that in 3 of 4 animals fed the deficient diet, fresh hemorrhages were seen microscopically in the adrenals. However, similar hemorrhagic lesions have been described in the adrenals of rats, due presumably to the lack of pantothenic acid (15).

CONCLUSIONS

1. Riboflavin is an essential dietary constituent for the pig.

2. Riboflavin deficiency in the pig is characterized clinically by retarded growth, corneal opacities, changes in the skin, hair and hoofs, and by a terminal collapse associated with hypoglycemia.

3. The chief findings at autopsy in 4 pigs fed the riboflavin deficient diet were as follows: changes of corneal epithelium in 4 animals, microscopic hemorrhages of adrenals in 3 animals, lipoid degeneration of proximal convoluted tubules in 2 animals, and lens cataract in 1 animal.

4. It is believed that certain of the changes observed, namely, spastic paralysis, anemia and possibly adrenal hemorrhages may have resulted from the lack of other food factors.

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THE RESPIRATION OF BROWN ADIPOSE TISSUE AND KIDNEY OF THE HIBERNATING AND NON-HIBERNATING GROUND SQUIRREL

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Since the discovery by Hoffmann and Wertheimer (1) that the adipose tissue consumes oxygen, a number of papers have been published on the metabolism of adipose tissue. They have shown that the tissue possesses, in addition to its mechanical protective and storage function, definite chemical activities.

The brown adipose tissue[1] found in many hibernating animals received only scanty attention, although it has been considered a gland of internal secretion, and a tissue which takes part in the hibernation process. Fleischmann (2) was the first to show that the oxygen consumption of the brown adipose tissue (rabbit, dormouse, hedgehog) was considerably higher than that of white adipose tissue. In order to study its rôle in hibernation it seems essential to know first its biochemical activities. These have been studied at the temperature of the animal in both the hibernating and the non-hibernating stages. This brown adipose tissue was found to have (when measured as fat-free tissue) as high a respiration as that of the kidney, to produce a large number of oxidations and to possess glycolytic activity. Furthermore, while the respiration of the liver and the kidney is considerably diminished at the temperature of hibernation, the respiration of the brown adipose tissue is diminished to a much less degree. In hibernation, therefore, while all the other tissues become quiescent, the brown adipose tissue retains a large proportion of its chemical activities.

EXPERIMENTAL. The animals used in these experiments were thirteen-lined ground squirrels obtained near Madison, Wisconsin, and Olympia Fields, Illinois. While at the laboratory they were fed standard rat ration. To induce hibernation the animals were kept without food or water for 48 hours, and then placed in a cold room at 5°. The animals were killed by a blow on the head, the axilar portion of the tissue was dissected rapidly,

[1] The brown adipose tissue has been called *hibernating gland, oil gland, brown fat, lipoid or cholesterin gland, organ of hibernation, hibernating mass, multilocular adipose tissue.*

the lymph nodes removed and thin sections made with a razor blade. The oxygen consumption and CO_2 production were measured with Barcroft-Warburg manometers with O_2 as gas phase in Ringer-phosphate of a final pH value of 7.31. The oxidizable substrates were used at a concentration of 0.01 *M*. The respiratory quotients were measured by the method of Warburg and Yabusoe (3). Anaerobic glycolysis was measured manometrically in Ringer-bicarbonate saturated with 5 per cent CO_2 plus 95 per cent N_2. When the effect of temperature on respiration was determined, the experiments were done simultaneously in two water baths, one kept in a room at 3°, the other in a room at 38°.

The oxygen consumption of brown adipose tissue. Fleischmann (2) reported that the brown adipose tissue of the newborn rabbit, the dormouse and the hedgehog consumed from 30 to 40 c.mm. of oxygen per 100 mgm. of wet weight. Felix and Eger (4) found about the same values for the brown adipose tissue of the rat. The brown adipose tissue of the ground squirrel showed greater oxygen consumption, the lowest figures obtained being 98 c.mm. and the highest 350 c.mm. Although the oxygen consumption, per milligram of dry weight, obtained in experiments with mixed sections of the tissue from two animals always gave figures reproducible within 10 per cent, the figures obtained in different months varied greatly. Since the fat content of the tissue was considered the main reason for this lack of uniformity, experiments were run where the oxygen uptake was determined per milligram of dry weight and per milligram of fat-free dry weight (the fat was extracted as usual by repeated extractions with ethyl ether and petrol ether up to constant weight). The oxygen uptake per milligram of dry weight varied from 2.3 c.mm. per hour to 10 c.mm., a difference of 334 per cent; the difference was reduced to 103 per cent for fat-free tissue (from 10.8 to 22 c.mm. O_2 uptake) (table 1). Other factors responsible for the variation seem to be a seasonal variation (the highest figures for oxygen uptake being found around September and October (table 2)), and the variable water content of the tissue (in 24 determinations of wet and dry weights the average ratio of wet weight : dry weight was 2.88 varying from 1.78 to 3.88).

The oxygen consumption of slices of brown adipose tissue had its optimum value when the solutions and gas phase were saturated with oxygen, and when the pH value of the Ringer-phosphate solution was 7.4. Respiration is considerably diminished when the tissue is ground.

In figure 1 are plotted the results of an experiment where the respiration of 100 mgm. of brown and white adipose tissue of the squirrel were measured simultaneously. The difference is obvious.

The respiratory quotient of white adipose tissue has been studied by a number of investigators. Felix and Eger (4) report that the R.Q. values rose from figures below 1 to values above 1 on addition of pyruvate, lactate,

and glycerol. The conclusion drawn from these experiments, namely, that this rise is evidence of synthesis of fat from carbohydrate, is untenable, because any one of the substances added may give different R.Q. values according to the manner in which the reaction of the particular substance is oriented. R.Q. values in *in vitro* experiments are only an indication of decarboxylation reactions accompanied by oxidation or reduction. The R.Q. value of the brown adipose tissue of the ground squirrel was about 0.80 (table 3). The R.Q. value of the tissue of an animal starved for eight weeks (with neither food nor water) went down to 0.67, presumably because of exhaustion of the tissue glycogen (Scoz (5), and Hoffmann and Wertheimer (1) have given figures for the glycogen content of adipose tissue).

TABLE 1

Oxygen consumption of brown adipos tissue of the ground squirrel

Temp. 38°; pH 7.31; buffer, Ringer-phosphate

O_2 UPTAKE PER HOUR		FAT CONTENT	RATIO OF DRY WEIGHT / FAT WEIGHT
Per mgm. dry weight	Per mgm. fat-free dry weight		
c.mm.	*c.mm.*	*per cent*	
3.3	15.8	79.5	4.9
2.9	16.2	81.7	5.5
4.6	12.4	62.9	2.7
10.0	21.2	52.7	2.1
8.7	21.2	59.2	2.5
6.5	22.0	70.6	3.4
6.1	18.1	66.3	3.0
6.5	22.0	70.6	3.4
2.3	11.8	70.8	5.3
2.9	10.8	73.2	3.7

TABLE 2

Oxygen consumption of brown adipose tissue at different months of the year

QO_2 represents average O_2 uptake in c.mm. per mgm. dry tissue per hour

MONTH	QO_2	
	Non-hibernating	Hibernating
May	4.9	
June	3.7	
July	3.5	3.85
August	4.4	3.0
September	6.4	
October	5.3	9.6
November	4.9	
December	5.9	9.8*

*Starved for 8 weeks.

The effect of temperature and hibernation on the respiration of brown adipose tissue and the kidney. Work on the respiration of hibernating animals has shown that during hibernation the respiration is considerably lowered. Fleischmann (2) pointed out that the difference was due solely to temperature, not to hibernation. Experiments with kidney slices of the ground squirrel showed similar results when no oxidizable substrate was added. However, some indication of functional damage of the kidney during hibernation was observed on studying the rate of oxidation of added substrates (pyruvate and succinate). The rate of oxidation of pyruvate by the kidney of the hibernating animal was diminished by 12 per cent; that of succinate by 18 per cent. These few experiments on oxidations produced by the kidney show that further work in this direction is needed to determine whether

there is some functional damage to tissue metabolism in hibernation (table 4). No difference was found in the respiration of brown adipose tissue whether alone or after the addition of succinate.

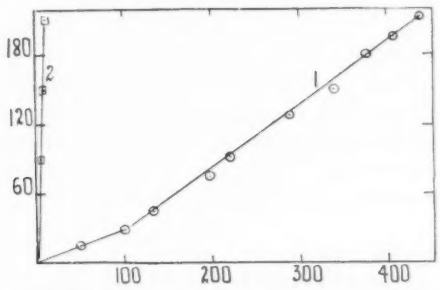

Fig. 1. Oxygen consumption of brown adipose tissue and omental fat of ground squirrel. T. = 38°; pH 7.33. Ringer-phosphate, buffer. Abscissa, O_2 uptake in cubic millimeters per 100 mgm. of wet weight of tissue; ordinate, time in minutes. *1*, O_2 consumption of brown adipose tissue; *2*, O_2 consumption of omental fat.

TABLE 3

Respiratory quotient of brown adipose tissue

Figures are given for O_2 uptake and CO_2 output per mgm. dry tissue per hour

OXYGEN UPTAKE	CO_2 OUTPUT	R.Q.
c.mm.	*c.mm.*	
4.6	3.5	0.76
4.9	4.2	0.85
3.3	2.9	0.88
3.95	3.25	0.82
5.74	4.47	0.78
6.8	5.0	0.73
*9.5	6.1	0.64
*9.4	6.65	0.71

* Starved for 8 weeks.

TABLE 4

Oxidations produced by the kidney of the ground squirrel in the hibernating and non-hibernating stages

Figures given are c.mm. O_2 uptake per mgm. dry weight per hour

SUBSTRATE	NON-HIBERNATING		HIBERNATING	
	38°	8°	38°	8°
None	15.5	2.2	15.6	2.5
Pyruvate	26.9	1.6	23.7	2.5
Succinate	39.5	2.2	32 3	1.7
Aceto-acetate	22.8	1.9		

That the brown adipose tissue plays some rôle during hibernation may be indicated by the striking difference that low temperatures (the body temperature of the hibernating animal) have on the rate of oxidation of the kidney and the brown adipose tissue. The respiration of the kidney diminished its oxygen consumption by 85 per cent; the respiration of the brown adipose tissue showed a diminution of 64 per cent. The same differ-

ence was found in the rate of oxidation of succinate; its oxidation by the kidney at 8° was inhibited completely while its oxidation by the brown adipose tissue was inhibited by 81 per cent (table 5). (Pyruvate and acetoacetate were not oxidized by the kidney at 8°.)

Oxidations produced by brown adipose tissue. Few studies have been made of the oxidations produced by adipose tissue. Felix and Eger (4) found that pyruvate, glycerol and lactate increased its oxygen consumption. Due to the variation from day to day of the amount of oxygen uptake, comparative rates of oxidation are given. They were obtained by taking as 100 the oxygen consumption of the tissue without added substrate. The tissue oxidized succinate at the highest speed, equal to that of the oxidation

TABLE 5

Effect of temperature on the oxygen consumption of the brown adipose tissue (B.A.T.), and the kidney of the ground squirrel

Figures given are c.mm. O_2 per mgm. dry weight per hour

SUBSTRATE	B.A.T.		KIDNEY	
	38°	8°	38°	8°
None	3.9	1.4	15.5	2.2
Succinate	10.0	1.85	39.5	2.2

TABLE 6

Oxidations produced by brown adipose tissue

Temp. 38°; buffer, Ringer-phosphate; pH 7.4. Figures express comparative rates when the O_2 uptake of the tissue alone is taken as 100

SUBSTRATE	COMPARATIVE RATE
Succinate	245
Pyruvate	145
Lactate	132
Butyrate	121
β-hydroxybutyrate	118
Alanine	118
Citrate	114
Glutamate	112
α-ketoglutarate	109
Glucose	100

of succinate by the kidney. Pyruvate, lactate, citrate, α-ketoglutarate, butyrate, β-hydroxybutyrate, dl-alanine and l (+) glutamate were also oxidized (table 6).

von Szent-Györgyi and his co-workers (6) found that small amounts of fumarate acted as catalysts of cellular respiration (C_4 dicarboxylic acid catalysis). The respiration of brown adipose tissue was not appreciably increased on addition of fumarate (0.0005 *M*) (table 7).

Effect of inhibitors. Ruska and Quast (7) ventured the opinion that the respiration of adipose tissue may not be an iron-porphyrin catalysis because they failed to find appreciable quantities of iron. As can be seen in table 8, the respiration of brown adipose tissue was almost completely inhibited

by HCN (0.0005 *M*) (96 per cent), an inhibition which never reaches such an extent in other mammalian tissues (8). It would seem that oxidations in this tissue proceed completely through iron-porphyrins. The respiration was also inhibited 71 per cent with 0.001 *M* arsenite, 61 per cent by 0.02 *M* malonate, and 53 per cent by 0.001 *M* iodoacetate.

Anaerobic glycolysis. The brown adipose tissue of the ground squirrel showed a measurable anaerobic glycolysis, both in the absence and in the presence of glucose. In the absence of glucose (autoglycolysis) there was 0.94 c.mm. CO_2 formation per milligram of dry weight (Q_L^N); in the presence of glucose the figure rose to 1.33. The Q_L^N values rose to 4.0 and 6.0 respectively when determined as fat-free dry tissue (table 9).

TABLE 7

Effect of fumarate on the respiration of brown adipose tissue

Temp. 38°; pH 7.4 (Ringer-phosphate). Concentration of glucose 0.01 *M*; of fumarate, 0.0005 *M*. Figures express O_2 uptake per mgm. dry weight

TIME	CONTROL	GLUCOSE	FUMARATE	GLUCOSE + FUMARATE
minutes				
30	5.0	4.5	5.2	4.9
60	9.0	8.0	9.4	8.5
90	12.6	11.0	13.7	11.8
120	16.1	14.8	17.6	15.0
150	19.5	18.0	22.0	18.4
180	23.0	21.7	25.6	21.8
210	26.6	25.3	30.1	26.0
240	31.0	30.0	34.5	30.2

TABLE 8

Effect of inhibitors on the respiration of brown adipose tissue

Temp. 38°; pH 7.4; Ringer-phosphate buffer

INHIBITOR	CONCENTRATION	INHIBITION
		per cent
Hydrocyanic acid..	0.005	96
Arsenite.....	0.001	77
Malonate.....	0.02	61
Iodoacetic acid....	0.001	53

Some components of enzyme systems. Methods. Cytochrome c was detected by the method of Keilin and Hartree (9). The extracted cytochrome was reduced with $Na_2S_2O_4$ and the absorption band at 5500 Å was observed with a Zeiss microspectroscope. A comparative determination of cytochrome oxidase was made as follows: 0.5 gram of tissue (heart and brown adipose tissue) plus 20 cc. H_2O was ground with sand, and the suspension was centrifuged to remove oxidizable substrates. To the residue were added 2 cc. of 0.06 *M* phosphate pH 7.0 plus 3 cc. H_2O; the suspension was poured into a mortar and ground once more with sand. A plane parallel optical cell containing 10 cc. of reduced cytochrome c (10 mgm. cytochrome dissolved in 12 cc. H_2O plus 2 cc. 0.1 *M* phosphate pH 7.0) was placed in front of a Zeiss microspectroscope. With the aid of a syringe 0.4 cc. of the tissue suspension was added rapidly, and the time of

disappearance of the 5500 Å absorption band was then recorded. Diphosphothiamine (cocarboxylase) was estimated by the method of Lohmann and Schuster (10). The beer yeast for these determinations was kindly furnished by the Keeley Brewing Company of Chicago. Diphosphothiamine was prepared at the laboratory. Ascorbic acid was determined by grinding the tissue with a mixture of trichloroacetic and metaphosphoric acids, the filtrate being titrated with 2,6-dichlorophenol indophenol (11).

The presence of cytochrome c was detected spectroscopically. The cytochrome oxidase content of the brown adipose tissue was 14 per cent that of the heart of the same animal. (With heart muscle the 5500 Å absorption band disappeared in 5 minutes, 10 seconds; with brown adipose tissue it disappeared in 35 minutes, 54 seconds.) The brown adipose tissue was also rich in diphosphothiamine; it contained 18 micrograms per gram of fresh tissue while the liver of the ground squirrel contained only 6.5 micrograms. After six weeks' hibernation there was a drop of only 15 per cent in both tissues (table 10). Ochoa and Peters (12) report that after thiamine deficiency the diphosphothiamine content in the liver dropped from 5.1 micrograms per gram to 0.44, i.e., there was a loss of 91 per cent. The ascorbic acid content was 0.111 mgm. per gram, one-third that found in the liver of the ground squirrel (0.375 mgm.).

TABLE 9

Anaerobic glycolysis of brown adipose tissue

Temp. 38°; buffer, Ringer-bicarbonate; pH, 7.4; glucose content, 0.01 *M*. Figures given are c.mm. CO_2 produced per mgm. dry weight per hour.

NO GLUCOSE	GLUCOSE
1.0	1.45
0.84	1.06
0.99	1.43
0.95	1.36
4.0*	6.0*

* Per mgm. fat-free weight.

TABLE 10

Some components of enzyme systems present in brown adipose tissue (brown squirrel)

COMPONENT	BROWN ADIPOSE TISSUE	OTHER TISSUE
Cytochrome c	++	Heart +++
Cytochrome oxidase	14	Heart (100)
Diphosphothiamine	15 γ × g†	Liver 5.5 γ
Diphosphothiamine	18 γ‡	Liver 6.5 γ
Ascorbic acid	0.111 mgm. × 1 gram	Liver 0.375 gram × gram

† Hibernating.
‡ Non-hibernating.

SUMMARY

The brown adipose tissue of the ground squirrel shows considerable metabolic activity when compared not only with the white adipose tissue

but with tissues of high metabolic activity; the O_2 consumption of the fat-free tissue was 17.1 ± 3.65 c.mm. per milligram of dry weight. The R.Q. was 0.80. The anaerobic glycolysis in the absence of glucose was 4.0 c.mm. CO_2; on addition of glucose it rose to 6.0. The tissue oxidized succinate and pyruvate with the same activity as the kidney; it also oxidized lactate, citrate, α-ketoglutarate, fatty acids, and amino acids. The respiration was almost completely abolished by HCN. The tissue contained cytochrome c, and the activity of its cytochrome oxidase was 14 per cent that of the heart. The diphosphothiamine content of the tissue was 18 micrograms per gram, three times that of the liver. The ascorbic acid content was 0.111 mgm. per gram, one-third that of the liver. When the oxygen consumption of the brown adipose tissue and the kidney was determined at the temperatures of hibernation and non-hibernation there was a striking difference; while the respiration of the kidney at the temperature of hibernation was only 15 per cent that of the respiration at 38°, the respiration of the brown adipose tissue was still 36 per cent. In hibernation, therefore, while all the other tissues reduce their metabolism to a minimum, the brown adipose tissue still retains one-third of its optimum activity.

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CERVICAL LYMPH PRODUCTION DURING HISTAMINE SHOCK IN THE DOG

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The following experiments were undertaken to determine whether, in the condition of shock, changes in lymph flow and composition might be found which would indicate alterations in the composition of the tissue fluid.

Low blood pressure, the most characteristic feature of the various types of shock, is generally agreed to be due to a reduction in the amount of blood available for circulation. This decrease in the effective blood volume may be brought about by any one or a combination of the following conditions: *a*, actual loss by hemorrhage; *b*, stagnation and pooling of blood in dilated peripheral vessels, and *c*, loss of fluid from the blood stream through increased capillary transudation. If the latter occurs to any great extent, the resulting accumulation of tissue fluid might promote an increased production of lymph.

An increase in the flow of thoracic duct lymph was observed in dogs during histamine shock (Dale and Laidlaw, 1911–1912) and during shock from intestinal manipulation (Mann, 1914). Thoracic duct flow is influenced so markedly by respiration and by the state of intestinal activity that it is difficult to evaluate data obtained from this source. Haynes (1932) reported an increase in the amount of leg lymph during short periods of histamine injection but states that, with one exception, her animals were not in a state of shock.

In a few of our preliminary experiments, shock was induced in anesthetized dogs by intestinal manipulation or by Best and Solandt's (1940) method of pulping the thigh muscle with blows from a rubber mallet. With both of these methods it was exceedingly difficult to control the time of onset and the degree to which shock was produced. It therefore seemed advisable to concentrate first on histamine shock, which is known to resemble traumatic shock in many respects (Dale and Laidlaw, 1918–1919) and has the advantage of being more easily controlled. In addition, it does not introduce complicating factors such as hemorrhage or the effects of wide variations between the time of trauma and the onset of shock—factors which alone might alter lymph production.

METHOD. In dogs anesthetized with nembutal (40 mgm. per kgm. of

body weight) the right and left cervical lymphatics and, in a few instances, the thoracic duct were cannulated. A continuous flow of cervical lymph was maintained by the "nodding head" technique (McCarrell, 1939). Artificial respiration was administered through a tracheal cannula; arterial pressure was recorded from the femoral artery and venous blood samples were removed from the opposite femoral vein. Venous pressure was determined at intervals with a saline manometer attached to a small glass tube, which was inserted through a cut in a side branch of the external jugular vein until the tip of the tube just reached the external jugular blood stream. During the operation and prior to the collection of lymph, physiological saline (20 cc. per kgm. of body weight) was given intravenously to insure adequate hydration of the tissues.

After a control period of approximately one hour, during which lymph was collected and the flow determined in milligrams per minute, the intravenous injection of histamine (4 mgm. of ergamine acid phosphate per cc. saline) was begun. The total amount of histamine injected depended on the sensitivity of the individual animal's blood pressure response and on the length of the experiment. The blood pressure was recorded constantly and sufficient histamine was given in repeated doses to maintain the blood pressure at 40 to 60 mm. of mercury. The blood pressure was kept at this low level from 1.0 to 6.3 hours, and the average dose of histamine was 2.8 mgm. per kgm. per hour. No nembutal was necessary during the period of shock. Changes in cervical lymph protein and serum protein were followed by refractometric determinations of protein percentage. Kjeldahl determinations were done on the thoracic duct lymph as it was generally too milky for accurate refraction. Very small amounts of venous blood were removed for oxygen determinations, and the removal had no noticeable effect on the blood pressure.

Results. Figure 1 is an example of responses noted during one typical experiment (dog 2), and table 1 contains figures for the results obtained in the series of eight experiments.

1. *Arterial pressure.* Within one minute after the beginning of the injection of histamine, the arterial pressure fell sharply in the manner described by Dale and Laidlaw (1918–1919) with slow histamine injections. This low pressure is due to a general dilatation of arterioles and capillaries and, in the dog, to the additional factor that blood is trapped in the splanchnic area by constriction of the hepatic veins (Best and McHenry, 1931). At the conclusion of the histamine administration the pressure, with one exception, rose to values slightly lower than the control levels but always above 90 mm. and usually above 100 mm. of mercury. In dog 6, however, the pressure remained at shock levels after histamine was discontinued, and the animal eventually died from the effects of the histamine.

2 *Cervical lymph flow.* A few minutes after the onset of the low arterial

pressure the cervical lymph flow increased, and peaks were quickly reached that were 1.1 to 4.9 times the control levels. As the period of shock

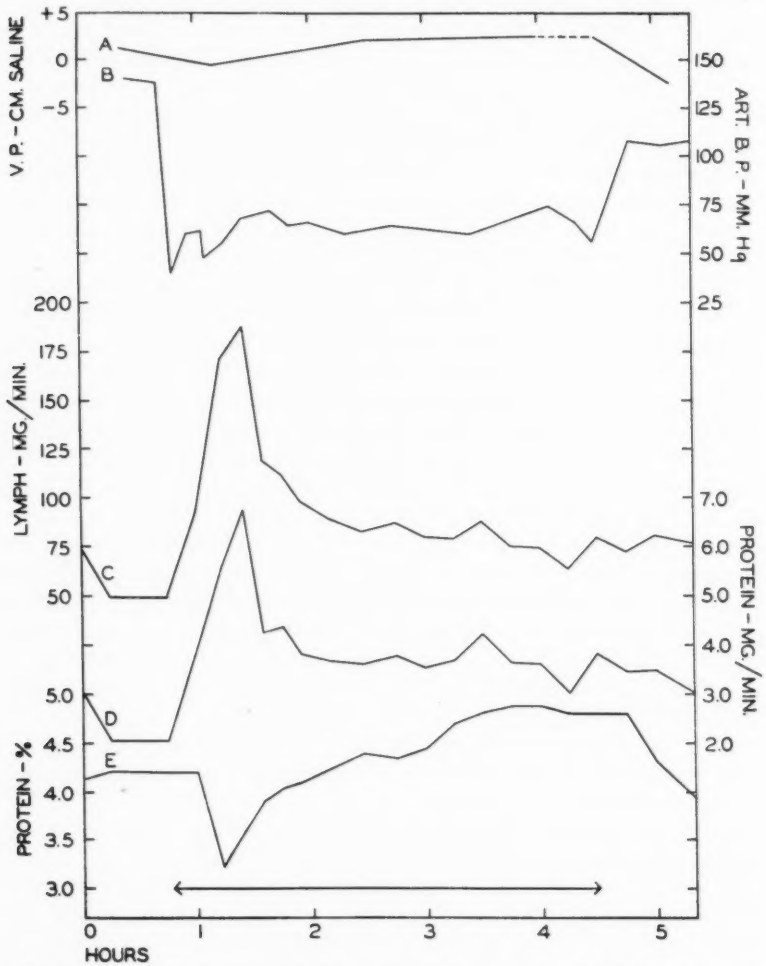

Fig. 1. Effect of intravenously injected histamine on cervical lymph production. *A*, venous pressure in centimeters saline; *B*, arterial pressure in millimeters of mercury; *C*, cervical lymph flow in milligrams per minute; *D*, cervical lymph protein in milligrams per minute; *E*, cervical lymph protein percentage. Period of histamine injection indicated by arrow.

continued the flow decreased from its peak, sometimes levelling off at or slightly above control values and sometimes continuing to decrease for the duration of the experiment. A small second rise was noted in a few cases

during the arterial pressure rise after the shock period, but this was not a consistent response. In experiment 1 (table 1) the arterial pressure was allowed to recover after being at a low level for one hour. During a subsequent period of shock the cervical lymph flow, which had decreased to

TABLE 1

Flow and characteristics of lymph in dogs given histamine shock

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	CERVICAL LYMPH			THORACIC DUCT LYMPH			AVERAGE ARTERIAL PRESSURE	VENOUS OXYGEN	SERUM PROTEIN	HISTAMINE
		Flow	Protein		Flow	Protein					
	kgm.	*mgm./min.*	*per cent*	*mgm./min.*	*mgm./min.*	*per cent*	*mgm./min.*	*mm. Hg*	*volumes per cent*	*per cent*	*mgm./kgm./hr.*
1	8.5	Control.... 77.6	2.44	1.89				171			2.4 for 1 hr.
		Shock.....154.2	2.35	3.62				62			
		Recovery . 59.0	2.34	1.38				144			
Repeat		Control... 53.2	2.32	1.23				145			2.5 for 1 hr.
		Shock..... 72.7	2.18	1.59				55			
		Recovery.. 54.3	2.33	1.27				137			
2	12.8	Control... 48.8	4.22	2.06				139			2.1 for 3.7 hr.
		Shock.....188.0	3.59	6.75				60			
		Recovery . 76.7	4.35	3.42				108			
3	8.5	Control.. 31.7	3.40	1.08				112	10.33	6.09	0.8 for 3 hr.
		Shock..... 82.3	3.25	2.67				50	3.09	5.23	
		Recovery . 23.4	3.46	0.63				93	3.49	5.42	
4	9.6	Control... 22.2	2.93	0.65				132	13.29	5.73	2.5 for 3.5 hr.
		Shock.... 85.5	2.52	2.16				52	18.20	5.10	
		Recovery . 18.0	2.84	0.51				112	19.64	5.04	
5	8.0	Control... 64.1	2.64	1.69				110	14.34	4.68	7.0 for 6.3 hr.
		Shock.....115.8	3.22	3.73				47	8.37	4.96	
		Not allowed to recover									
6	9.0	Control... 4.6	3.70	0.13	139.4	3.83	5.35	128	20.08	4.96	3.0 for 2.4 hr.
		Shock..... 11.7	3.10	0.36	238.5	5.29	12.62	37	9.69	5.32	
		Died in shock									
7	6.8	Control... 21.1	3.38	0.72	169.0	3.29	5.56	127	17.72	4.81	3.9 for 2.9 hr.
		Shock..... 24.2	3.08	0.75	740.0	4.47	33.10	50	10.00	5.68	
		Recovery . 24.7	3.04	0.75	10.5	4.44	0.47	96	15.87	5.06	
8	8.9	Control... 31.8	4.18	1.33	370.0	4.29	15.90	137	23.32	5.73	1.0 for 2.5 hr.
		Shock.....152.2	2.63	4.08	388.0	4.88	18.98	55	15.06	5.38	
		Recovery . 25.0	3.43	0.86	162.0	4.87	7.89	122	17.74	5.17	

approximately the control value, again rose. This second peak was not as great as the peak obtained during the first shock period.

3. *Cervical lymph protein.* The lymph protein percentage in all but one case decreased with the rise in lymph flow. The flow was so large that, in spite of its reduced protein percentage, the protein in the lymph in milligrams per minute was in the majority of the cases markedly increased.

As the flow decreased, following its peak, the protein percentage rose to approximately control values.

4. *Thoracic duct lymph.* In two of the three experiments in which the thoracic duct was cannulated there was a large rise in flow, percentage of lymph protein, and protein in milligrams per minute at the onset of the shock period and coincident with the rise in cervical lymph flow. The thoracic duct changes will not be discussed in detail since the increased intestinal motility and constriction of hepatic veins, which histamine is known to cause (Best and McHenry, 1931), introduce complicating factors that undoubtedly influence thoracic duct flow to a greater degree than do changes in capillary permeability.

5. *Serum protein percentage.* In three experiments the serum protein percentage rose and in three others the percentage decreased during the period of histamine shock. Derer and Steffanutti (1930), Atchley, Richards and Benedict (1931), and Haynes (1932) found no change or a slight fall in serum protein per cent after histamine administration, while Beard, Wilson, Weinstein and Blalock (1932) report a slight increase.

6. *Venous pressure.* There were no consistent or significant changes in venous pressure during the experiments. Control values averaged 1.8 cm. of saline. In four instances the pressure rose 0.4 to 5.5 cm. and in five cases dropped 1.3 to 6.9 cm. during the period of shock.

7. *Venous oxygen content.* In all but one experiment (no. 4) the oxygen content of the venous blood was significantly lower during and after the period of shock as compared with the control period. This is an expression of the slow capillary circulation and stagnation that occurs during shock and is in accord with Aub and Cunningham's (1920–1921) studies on traumatic shock in cats. They found not much change in the oxygen content of arterial blood, but a diminished oxygen content of venous blood was still present after the apparent recovery from shock. Aub and Cunningham give this as evidence for the existence of tissue anoxemia during and after shock conditions.

Discussion. Dale and Laidlaw (1918–1919) emphasized the fact that the reduction in circulating blood volume so characteristic of histamine shock is brought about mainly by capillary dilatation, but that this condition is augmented by a loss of plasma through damaged capillary walls. Additional evidence has been reported for an increase in the transudation of plasma (Atchley, Richards and Benedict, 1931) and for a loss of water from the blood (Butler, Beard and Blalock, 1931) during histamine shock in dogs.

The present analysis of lymph production in a subcutaneous area gives clear evidence that early in the period of histamine shock the capillaries become more "leaky" and a large amount of proteinized fluid escapes from the blood stream and may be recovered as lymph. However, this

state of increased capillary permeability is evidently not sustained throughout the period of histamine shock. Capillary conditions apparently become more or less stabilized quite soon, and factors such as an increased osmotic pressure of the blood or a decreased filtration pressure must inhibit fluid transudation to such an extent that lymph production is not permanently increased.

Since it is very possible that anoxemia plays an important rôle in any condition in which capillary blood flow is diminished, it is interesting to note that Maurer (1940), in experiments on the effects of anoxemia on cervical lymph flow, found somewhat the same temporary quality in the increased flow brought on by a period of low oxygen ventilation.

SUMMARY

Cervical lymph flow and protein content were studied in dogs during histamine shock in order to obtain evidence relative to the state of capillary permeability during this condition. Blood pressure was reduced to 40 to 60 mm. of mercury for periods of from 1.0 to 6.3 hours by intravenous doses of histamine. Early in the period of shock a considerable amount of proteinized fluid escaped from the blood stream, as was shown by cervical lymph flows that were 1.1 to 4.9 times the control values. The lymph protein percentage became less but the amount of protein in milligrams per minute was markedly increased. This condition of increased capillary transudation was not permanent for, as the period of shock continued, lymph flow and protein content returned approximately to their normal values.

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THE CIRCULATORY RESPONSES OF NORMAL AND SYMPATHECTOMIZED DOGS TO ETHER ANESTHESIA[1]

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When ether is administered to normal animals, the following circulatory changes occur: abdominal vasoconstriction (1), cerebral vasodilatation (2), and probably dilatation of the cutaneous vessels (3). In the dog, there is also evidence of an increased blood flow in the deep vessels of the lower extremities (4). These adjustments are so balanced that even in the presence of an increased cardiac output (5, 6), ether anesthesia results in little or no change in the mean arterial pressure. Such precise regulation suggests a high degree of autonomic nervous integration. In view of this, we have attempted to evaluate the rôle of the sympathetic nervous system by studying the circulatory responses to ether inhalation which are shown by completely sympathectomized dogs.

METHODS. All operations were carried out aseptically under nembutal anesthesia (30 mgm. per kilogram of body weight, intravenously). The sympathectomies were usually done in two stages (7), although in two animals the three-stage operation was used (8). In most of the dogs, the completeness of the sympathectomy was verified by autopsy. Since the sympathetic chains were usually removed intact, and since the formation of dense scar tissue invariably obscured the original site of the nerve chains, we do not attach much significance to these gross examinations. The carotid arteries of three sympathectomized dogs were enclosed in skin flaps so that they could be occluded easily. The absence of any significant increase in blood pressure when the carotid arteries were occluded served as a functional test for the completeness of the sympathectomy (9). It must be emphasized that these animals were in good condition when used for experimentation. Most of them had returned to their preoperative weight, and several had gained weight.

In two sympathectomized dogs, the right vagus was severed of all its connections in the lower cervical region except the recurrent laryngeal

[1] A preliminary report of this work was presented before the American Physiological Society, New Orleans. This Journal **129:** P449, 1940.

nerve, and the left vagus was enclosed in a skin flap in the same fashion as the carotid arteries. The intact left vagus was sectioned under local anesthesia (2 per cent novocain) when the ether experiment was carried out. This procedure was adopted because it was our experience that chronically vagotomized preparations either died or became too sick from vomiting to permit further work.

The carotid sinus functions were abolished in two dogs by stripping the carotid arteries at their bifurcations and for a considerable distance along each branch. The absence of a rise in blood pressure when the carotid arteries were occluded demonstrated that the carotid sinuses had been completely denervated (10).

Ten days to two months after the final operation, the sympathectomized dogs were placed upon the animal board for a control period of one and one-half to two hours. Ether was then administered by the drop method for one hour.

The mean arterial blood pressure in the femoral artery was determined directly by arterial puncture, or by the insertion under local anesthesia (2 per cent novocain) of the usual glass cannula, attached to a mercury manometer.

The heart rates were counted from the kymograph records and checked against rates counted with a stethoscope.

Blood ether concentration was measured by a modification of the ordinary iodine pentoxide method (20). Since it is generally conceded that jugular vein blood yields the best approximation of the ether concentration in the brain, blood from this source was used for the ether determinations. Control experiments were performed on ten normal dogs.

Results. In describing the following experiments only two stages of anesthesia have been recognized: that of excitement and that of surgical anesthesia. The events occur so quickly in the dog that it is impractical to distinguish the first and fourth stages.

The mean femoral arterial pressure in ten resting, unanesthetized, normal dogs averaged 120 mm. Hg, and the heart rate averaged 95 beats per minute. The induction of ether anesthesia (fig. 1) produced an immediate and considerable rise in blood pressure accompanied by a pronounced cardiac slowing. As the stage of excitement was passed and the stage of surgical anesthesia was entered, the blood pressure returned towards the preanesthetic level and the heart rate increased markedly. Under full surgical anesthesia, with blood ether values between 100 and 150 mgm. per cent, the mean arterial pressure was generally 10 to 15 mm. Hg lower than the control level. In this stage, the heart rate usually varied from 160 to 205 beats per minute. Occasionally rates as low as 120 and as high as 270 were observed.

Thirteen experiments on eight completely sympathectomized dogs

showed an average control blood pressure of 110 mm. Hg. The heart rate averaged 75 beats per minute. With the first breath of ether (fig. 2), there occurred an immediate fall in blood pressure, amounting in some

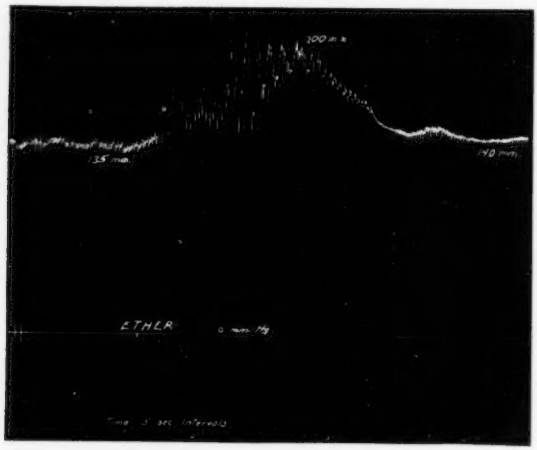

Fig. 1. The effect of ether inhalation upon the blood pressure of the normal dog. Note the bradycardia and the rise in blood pressure.

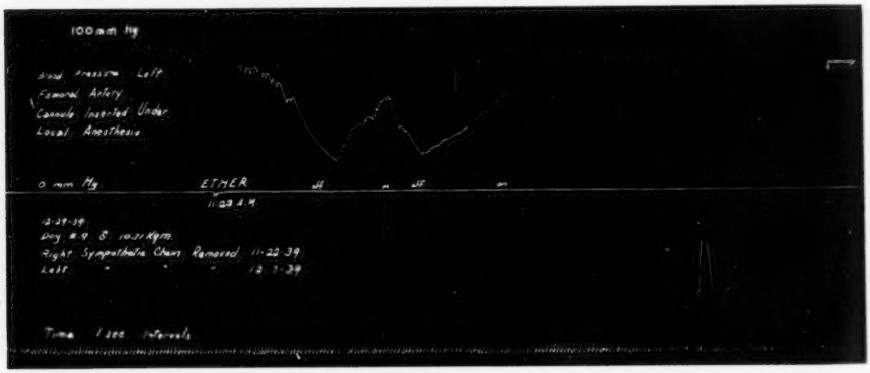

Fig. 2. The effect of ether inhalation upon the blood pressure of the totally sympathectomized dog. Note the periods of asystole which disappear when ether is removed at "off."

animals to as much as 80 mm. Hg. At the same time there was a pronounced bradycardia, and in some animals the heart ceased beating altogether. So sudden and so drastic were these changes that three dogs were killed by only three or four inhalations of ether. In one instance, death

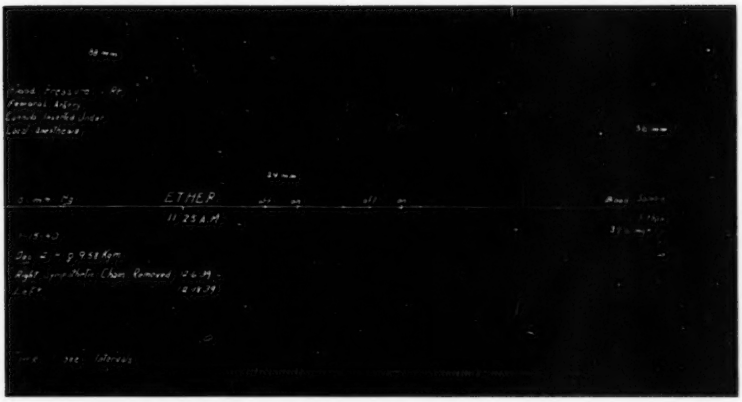

Fig. 3. The effect of ether inhalation upon the blood pressure of the totally sympathectomized dog. The blood pressure did not rise when the heart rate accelerated to the extent that it did in the experiment shown in figure 2.

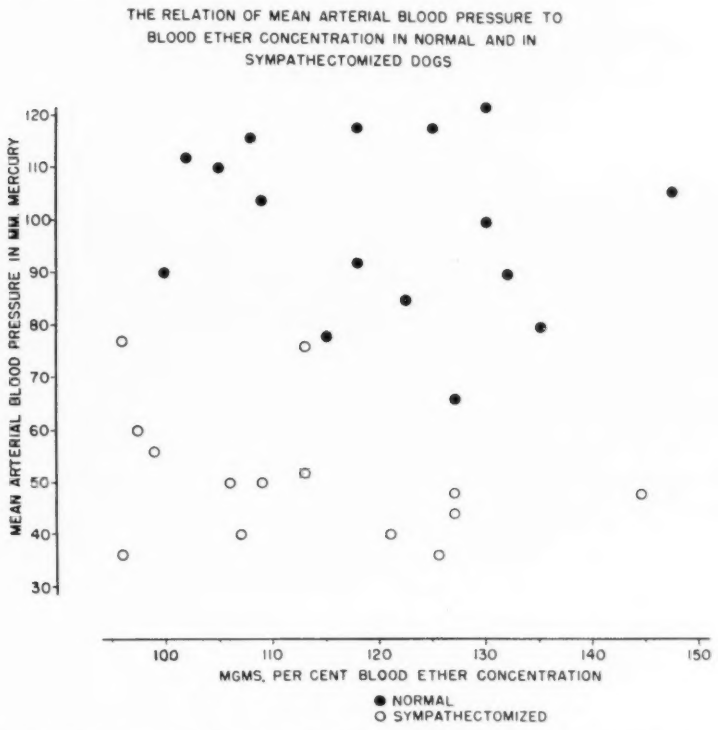

Fig. 4. The blood pressure values shown in the chart correspond to the pressures which were present when the ether samples were taken. They do not necessarily represent the average blood pressure values during anesthesia.

occurred with a blood ether concentration of 43 mgm. per cent. Such unfortunate accidents were avoided by removing the ether cone during the periods of asystole (see fig. 2). This manoeuver resulted in an immediate rise in blood pressure, at which time it was again safe to administer ether. As anesthesia deepened, the heart no longer responded with these critical periods of asystole, and with a rising arterial pressure, the animal passed through the period of induction. In some experiments, the return of the blood pressure towards the control value was striking (fig. 2), but in others (fig. 3) it remained at a low level. As muscular tone disappeared

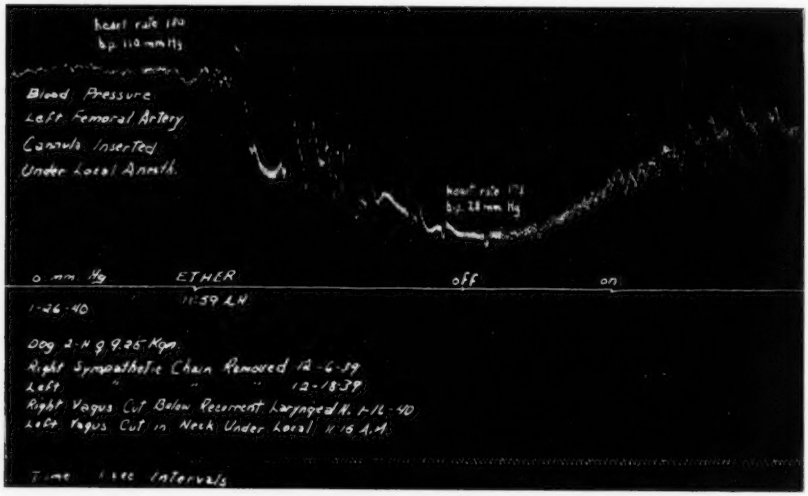

Fig. 5. The effect of ether inhalation upon the blood pressure in a vagotomized, sympathectomized dog. The high heart rate shown by this animal is related to the acute section of the left vagus nerve. The denervated heart rate in dogs kept for several days was always between 128 and 135 beats per minute.

and complete surgical anesthesia was attained, the blood pressure usually ranged between 30 and 70 mm. Hg (fig. 4). In this stage the heart rate of sympathectomized dogs averaged 132 beats per minute, with a range of 128 to 135. It should be noted that sympathectomized dogs could be maintained in the surgical stage of anesthesia with lower blood ether values than could normal dogs.

The circulatory response to ether inhalation was studied in three sympathectomized, vagotomized dogs. In every experiment the administration of ether caused a prompt fall in mean blood pressure without any gross change in cardiac rate (fig. 5). Since there were no periods of asystole

during induction, surgical anesthesia was attained more easily. Otherwise, the circulatory reactions were similar to those shown by sympathectomized dogs with intact vagi.

One sympathectomized dog was injected subcutaneously with atropine sulfate (1.0 mgm.). Twenty minutes later the heart rate had increased to 230 beats per minute. This value, which is far in excess of the rate of the denervated dog heart (132 per min.), has been attributed to the central stimulation of the vagal cardio-accelerator fibers (11). The inhalation of ether by the atropinized, sympathectomized dog resulted in a prompt fall in blood pressure without cardiac slowing.

The functions of the vagi and of the carotid sinuses were eliminated in two dogs. The administration of ether to these animals produced a greater rise of blood pressure during the induction stage than was observed in the

TABLE 1

DOGS	STAGE OF ETHER ANESTHESIA			
	Excitement		Surgical	
	Heart rate	Blood pressure	Heart rate	Blood pressure
Normal	Slowed	Elevated	Accelerated	Unchanged ± 15 mm. Hg
Sympathectomized	Slowed	Reduced	Accelerated to denervated heart rate	Reduced
Sympathectomized, vagotomized	Unchanged	Reduced	Unchanged	Reduced
Buffer nerves cut: carotid sinuses denervated and bilateral vagotomy	Unchanged	Elevated	Accelerated	Reduced to level of normal, etherized dog

records of normal dogs. No periods of asystole were present during the excitement stage, but some cardiac acceleration was noted when the anesthesia deepened. In the stage of surgical anesthesia, the blood pressure was maintained at or above normal levels.

The effects of ether anesthesia upon the heart rate and the blood pressure of the various animals used in this study are summarized in table 1.

DISCUSSION. The bradycardia shown by normal and sympathectomized dogs during the excitement stage of ether anesthesia was absent in vagotomized dogs and in dogs in which the vagal cardiac impulses had been blocked by atropine. This vagal effect is not the result of a carotid sinus reflex secondary to the rise in systemic blood pressure, for it occurred in sympathectomized dogs in which the blood pressure fell as well as in ani-

mals with denervated sinuses. The slow heart rate can be explained as a reflex stimulation of the vagal centers induced by the irritant action of ether upon the respiratory mucosa (12).

The rapid heart rate of the surgical stage of ether anesthesia is probably the result of stimulation of the cardio-accelerator mechanism and of inhibition of the vagal mechanism (13, 14). That vagal release occurs is shown by the fact that the heart rate is increased to about 132 beats per minute in etherized, sympathectomized dogs. The failure of the heart rate of such animals to exceed that of the denervated heart indicates that the vagal accelerator fibers are not excited by ether.

It is not clear from our experiments that the adrenal secretion which is said to occur during ether anesthesia (15) plays an important rôle in the changes in heart rate. The administration of ether to two dogs with inactivated adrenals showed that the usual cardiac acceleration was present. That the denervated gland is not affected directly by ether is demonstrated by the observation that the inhalation of ether by vagotomized, sympathectomized dogs produced no changes in the heart rate. These findings indicate that changes in the heart rate during ether anesthesia can be accounted for on a neural basis.

In the normal dog the excitement stage of ether anesthesia was associated with a rise in arterial pressure. This is in marked contrast with the response of the sympathectomized animal which, under these conditions, showed an abrupt fall in mean blood pressure. During surgical anesthesia (blood ether, 100 to 150 mgm. per cent), the mean arterial blood pressure of the normal dog was maintained between 100 and 120 mm. Hg. After complete sympathectomy, the blood pressure at corresponding blood ether levels was low (30 to 70 mm. Hg), and the pressure was almost inversely proportional to the blood ether concentration. These observations demonstrate that the sympathetic nervous system is essential for the blood pressure responses to ether which are shown by the normal dog.

The sympathetic vaso-constrictor impulses responsible for these reactions may arise from reflex stimulation of the vasomotor center. It is conceivable that certain blood ether concentrations may produce peripheral vaso-dilatation which in the normal animal is masked by vascular reflexes originating in the aorta and in the carotid sinuses. Since the blood pressure was maintained at or above normal levels when ether was administered to dogs with the circulatory buffer nerves cut, it may be concluded that the sympathetic vaso-constrictor mechanism of etherized, normal dogs functions in the absence of afferent impulses from the aorta and carotid sinuses.

The possibility of a direct stimulation of the vasomotor center by ether was considered by Pilcher and Sollmann (16) who concluded that any vasomotor stimulation observed during ether anesthesia is the result of

anoxemia induced by respiratory depression. Determinations of the arterial oxygen content and oxygen capacity (Shaw and Downing's (17) modification of the Van Slyke-Neill method) were made upon normal and sympathectomized dogs. The striking difference between the vascular responses of our normal and sympathectomized dogs bore no relation to the degree of oxygen unsaturation. From this it appears that during ether anesthesia sympathetic vasoconstriction is not necessarily dependent upon the presence of anoxemia.

During the induction stage of ether anesthesia, the blood pressure of sympathectomized dogs falls. The fall in blood pressure appears whether there is a marked cardiac slowing (sympathectomized dogs with intact vagi, figs. 2 and 3), or whether the heart rate remains unchanged (vagotomized, sympathectomized dogs, fig. 5). It must be noted, however, that pronounced vagal slowing aggravates the fall in pressure, and increases the danger of fatality. The sudden fall in blood pressure which occurs when a sympathectomized animal struggles (18, 19), is not identical with the blood pressure response to ether inhalation. This is shown by the fact that during ether induction the arterial pressure frequently falls 40 to 60 mm. Hg before actual struggle has begun. The relation of this fall in blood pressure to the posterior root dilators is under investigation.

Since there are no data concerning the effect of ether upon the cardiac output of the sympathectomized animal, it is not possible to evaluate the factors which produce the low blood pressure shown by etherized, sympathectomized dogs.

The emergency function of the sympathetic nervous system of the cat has been recognized for some time. Recent studies upon sympathectomized dogs have failed to demonstrate a similar homeostatic function in this species (21). The above experiments are, therefore, of considerable interest, for they are among the first to indicate the importance of the sympathetic nervous system of the dog in the preservation of homeostasis.

This investigation resulted from conversations with Dr. M. I. Gregersen for whose continued interest and support we are grateful.

SUMMARY

1. In normal dogs the induction of ether anesthesia produces, during the excitement stage, a rise in blood pressure accompanied by bradycardia. Under surgical ether anesthesia the mean arterial blood pressure is usually 10 to 15 mm. Hg lower than the control blood pressure. In this stage the heart rate increases to between 160 and 205 beats per minute.

2. The administration of ether to completely sympathectomized dogs produces an immediate fall in blood pressure to between 40 and 70 mm. Hg. This is associated with a marked bradycardia. During surgical anesthesia,

the heart rate increases to about 132 beats per minute. The blood pressure remains low, and varies inversely with the blood ether concentration.

3. The inhalation of ether by vagotomized, sympathectomized dogs is attended by a prompt fall in blood pressure without any gross change in heart rate.

4. When ether is administered to dogs with the vagi and carotid sinus nerves cut, the blood pressure increase is greater than that shown by normal animals. During surgical anesthesia, the blood pressure is maintained at or above normal levels.

5. It is concluded that the function of the sympathetic nervous system is essential for the maintenance of the blood pressure at normal levels during ether anesthesia.

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THE LYMPH DRAINAGE OF THE GALL BLADDER TOGETHER WITH OBSERVATIONS ON THE COMPOSITION OF LIVER LYMPH

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While working upon problems of lymph movement in the upper part of the abdomen, we were struck with the extraordinary number and size of the lymphatic trunks upon the surface of the gall bladder of the cat. It proved easy to cannulate these vessels and collect what was certainly gall-bladder lymph, but when this was done two facts emerged: first, the amount of lymph collected per minute was very large; and second, the protein concentration of the lymph was practically that of the blood.

An examination of the literature showed that Sappey (1874), for human material, had pictured lymphatics passing from the liver lobes over to join draining trunks upon the surface of the gall bladder, and he left no doubt that gall-bladder lymph and liver lymph must be very closely related. Sudler (1901) examined the situation in the dog, cat, pig and man, and came to a similar conclusion as to the communication of gall-bladder and liver lymphatics. In 1927 Winkenwerder made retrograde injections of gall-bladder lymphatics in cats, and stated there were no direct connections between gall-bladder and liver vessels. This conclusion is not correct for the cat, dog, monkey and rabbit, and in all probability will not hold for man, though since the work of Sappey (1874) there are no direct observations upon human material.

EXPERIMENTAL. In all cases nembutal anesthesia has been used, and most of the observations have been made upon cats. The gall bladder was exposed through a small incision in the upper abdominal wall, and one of the large lymphatic trunks in the gall-bladder wall was cannulated. Through the same incision it was easy to cannulate a lymphatic coming directly from the liver. Table 1 shows the rates of lymph flow from the gall-bladder vessel, which are very high, together with protein concentrations in blood, in liver and in gall-bladder lymph. The identity of the three fluids is quite clear and is made more definite through table 2, which shows the extraordinary similarity in protein content of blood serum, liver and gall-bladder lymph.

Such figures as these, coupled with our knowledge of the large amount

TABLE 1

Lymph flow from a gall-bladder lymphatic in the cat with protein concentrations in liver lymph, gall-bladder lymph and blood serum

NUMBER OF ANIMAL	LYMPH FLOW FROM GALL BLADDER	PROTEIN CONCENTRATIONS		
		Gall-bladder lymph	Liver lymph	Blood serum
	mgm. per min.	*per cent*	*per cent*	*per cent*
1	11.2	7.00	6.88	7.19
2	54.1	6.88	5.36	6.05
3	21.5	5.64	6.01	5.81
4	49.2	5.15	5.05	4.36
5		6.97	6.58	7.35
6	41.2	4.90	5.03	5.19
7		5.38	5.44	5.33
8	90.7	5.27	5.31	5.29
9		4.16	5.07	5.29
10		4.74	5.03	5.57
Average		5.61	5.58	5.74

TABLE 2

Protein fractionation in blood, liver and gall-bladder lymph in two cats

NUMBER OF ANIMAL	GALL-BLADDER LYMPH			LIVER LYMPH			BLOOD SERUM		
	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
8	5.27	3.47	1.80	5.31	3.39	1.92	5.29	3.37	1.92
10	4.74	2.88	1.86	5.03	2.91	2.12	5.57	3.38	2.19

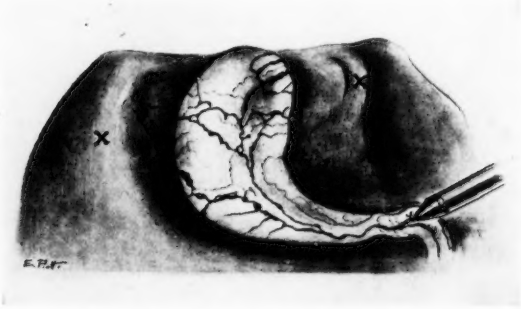

Fig. 1. Exposure of gall-bladder lymphatics in a cat by means of a dilute solution of India ink injected into the liver substance. The cannula is in one of the large draining trunks easily seen on the surface of the bladder.

Note: After this paper was submitted for publication, a drawing similar to our own was found in H. Baum's *Das Lymphgefässsystem des Hundes*, published by August Hirschwald, Berlin, 1918, showing clearly the connections between gall-bladder and liver lymphatics in the dog.

and high protein content of lymph coming from the liver, made it probable that liver lymphatics joined gall-bladder vessels very freely, and this fact was readily demonstrated by injections. Figure 1 is a drawing of the gall-bladder lymphatics in a cat. This animal was injected with a dilute solution of India ink at the two points marked *X*, the injection mass being simply forced into the liver substance and being recovered not only from the liver lymph but also from the lymph leaving the gall bladder.

Similar observations have been made in rabbits, dogs and monkeys, so that there can be no doubt of intimate connection of liver and gall-bladder vessels. This relation may be of great importance in gall-bladder infections, since the free flow of liver lymph would readily accelerate absorption from a pathological gall bladder.

DISCUSSION AND CONCLUSIONS. There can be no question as to the connection of gall-bladder and liver lymphatics. It is free and extensive. The composition of the two lymphs is identical, and injections into the liver substance are readily traced into the gall-bladder lymphatics. These facts have been demonstrated for the cat, rabbit, monkey and dog, the freedom of communication being in the order of the listing of the species. A final fact marches with them, namely, the similarity in protein content of serum and liver lymph as brought out in table 1 and particularly in table 2. These findings can mean but one thing, namely, that the liver cells are bathed in blood plasma, and in this respect are perhaps in a unique position in the mammalian body, the situation in the spleen being the only one which has the probability of similarity.

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CONCENTRATION OF ASCORBIC ACID AND THE PHOSPHATASES IN SECRETIONS OF THE MALE GENITAL TRACT[1]

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Since there are high concentrations of ascorbic acid and of phosphatases whose optimal activity occurs at weakly acid and at alkaline pH, in the semen of certain species, this investigation was carried out to determine the loci of origin and the interrelationship of these substances in the fluids of the genital tract of human, dog and guinea-pig males. Farrell (1938) observed continuous secretion of the prostate gland in dogs and Mislawsky and Bormann (1899) discovered that prostatic secretion was greatly augmented by parenteral injection of pilocarpine; in the present study a comparison was made of the principal electrolytes and of phosphatase content of "resting semen" excreted without adventitious stimulation and of pilocarpine-stimulated semen in dogs.

Zimmet and Sauser-Hall (1936) found between 4.6 and 6.4 mgm. of ascorbic acid in 100 grams of normal guinea-pig ejaculate. Zimmet (1939) studied the ejaculate of guinea pigs in experimental scurvy and at the end of 3 weeks found the ejaculate was abnormal in that it failed to coagulate and did not contain sperm, and that the ascorbic acid content was reduced to 0.8 mgm. per cent; the content of ascorbic acid was doubled in 3 hours following subcutaneous injection of 50 mgm. of this substance. Nešpor (1939) found 2.6 to 3.4 mgm. of ascorbic acid per 100 cc. of human semen. Phillips, Lardy, Heizer and Rupel (1940) observed ascorbic acid concentrations of 3 to 8 mgm. in 100 cc. of normal bull semen; levels below 2 mgm. were associated with poor breeding activity. In every case the concentration of ascorbic acid in semen was higher than that of blood plasma. No study of the source of ascorbic acid in semen has been published.

In addition to the phosphatase with optimal activity at pH 9, Davies (1934) and Bamann and Riedel (1934) discovered another phosphatase with optimal activity at pH 5 in extracts of spleen, liver and kidney. Kutscher and Wolbergs (1935) observed that acid phosphatase is present

[1] This investigation was aided by a grant from the Committee on Research in Problems of Sex of the National Research Council.

in human ejaculate and in extracts of prostate gland in higher concentrations than any phosphatase in any other biological material. Kutscher and Pany (1938) found that the prostate gland of the dog contains considerably less acid-phosphatase than the human gland.

METHODS. Prostatic fluid was collected from 20 normal male dogs by the method of Huggins, Masina, Eichelberger and Wharton (1939). Both the natural excretion of the genital tract obtained without using any external stimulant, *resting semen*, and the *stimulated semen* collected following intravenous injection of pilocarpine hydrochloride, 6 mgm., were studied; in the dog the prostate gland contributes most of the volume of semen. Water determinations were made by drying known weights of these fluids to constant weight at 105°C. Chloride, pH and total CO_2 were determined by methods cited in the last mentioned paper. Specific gravity was measured by the falling drop method of Barbour and Hamilton (1926). Phosphatases were determined by the method of King and Armstrong (1934) utilizing disodium monophenyl phosphate as substrate and 0.2 N sodium acetate-acetic acid at pH 5 as buffer for the acid-phosphatase and 0.2 M sodium barbital at pH 9 as the buffer for alkaline-phosphatase determination. The results correspond to King and Armstrong units per 100 cc. of fluid.

Ascorbic acid determinations were made on ejaculate from 20 normal adult guinea pigs, on seminal vesicle fluid from 13 and on the prostatic fluid of 7 of these animals. Ejaculate was obtained by electrical stimulation according to the method of Battelli (1922). Thick granular seminal vesicle fluid was secured by surgical removal of these appendages; the removal was done as completely as possible and the wound repaired. After 7 days, electrical ejaculation was carried out and between 0.2 and 0.4 gram of thin watery fluid was obtained. When this fluid contained coagulated particles, it was considered to be grossly contaminated by seminal vesicle contents and the animal was discarded; otherwise, the fluid was regarded as consisting chiefly of prostatic fluid. All fluids were collected in tared stoppered bottles. Ascorbic acid determinations were made on normal human semen obtained by ejaculation into a glass bottle, from 7 normal men. In addition, using digital pressure per rectum on the appropriate gland, prostatic fluid was obtained from 11 men, seminal vesicle fluid from 3, and a mixture of seminal vesicle and prostatic fluid from 7 men. The methods of identification of these fluids were those of Huggins and Johnson (1933). Spermatocele fluid was obtained from 6 men. All fluids were assayed within a few minutes of collection, the longest elapsed time being about 30 minutes after collection.

Ascorbic acid determinations were made by titration with 2,6-dichlorophenol indophenol as described by Bessey (1939). All extractions and dilutions were made with 3 per cent metaphosphoric acid in water.

RESULTS AND DISCUSSION. Resting semen was excreted in amounts, 0.1 to 2 cc. per hour, in the normal adult dog; it was slightly more turbid than the opalescent fluid obtained by pilocarpine stimulation, the increased turbidity apparently being due to the fact that it contained about twice as many epithelial cells and leukocytes; there were fewer spermatozoa in resting semen than after pilocarpine injection. The specific gravity of both resting and stimulated centrifuged semen varied from 1.0057 to 1.0082. Stimulated semen was always about 0.6 pH unit more acid, and the CO_2 content was 0.7 to 0.9 mM higher than resting semen. The chief difference between these fluids (table 1) lies in the chloride content, the chlorides of resting semen being considerably lower than those of semen obtained after pilocarpine stimulation. Ball (1930) found that the sum of bicarbonate and chloride ions in pancreatic juice is nearly constant

TABLE 1

Normal values for resting semen of dogs collected without external stimulation and semen obtained following pilocarpine injection

Values expressed per liter of fluid

CONSTITUENT	RESTING SEMEN			PILOCARPINE STIMULATED SEMEN		
	Number of determinations	Mean	Standard deviations	Number of determinations	Mean	Standard deviations
pH	20	6.72	±0.33	15	6.14	±0.19
CO_2mM	10	1.23	±0.2	10	2.05	±0.27
Chloride, m.-eq.	23	104	±22.6	15	156	±6.0
Water, grams	10	981	±4	17	981	±3

regardless of the rate of flow, but no such relationship was observed by us in semen.

In the prostatic fluids of 17 dogs, acid phosphatase varied from 3 to 286.5 units, and alkaline phosphatase from 0 to 106.75 units per 100 cc. Pilocarpine stimulation caused an increase of acid phosphatase and a decrease of alkaline phosphatase in stimulated semen as compared to resting semen. This reciprocal relationship of these enzymes (table 2) constantly occurred. The intracellular distribution of alkaline-phosphatase has been studied by Gomori (1939) who found the chief localization in the prostate of this enzyme around the capillaries, only traces being present in the epithelial cells. By a new histochemical method, which demonstrates acid-phosphatase, Gomori (1941) found large amounts of this substance in the epithelial cells. The present findings show that on stimulation with pilocarpine, acid-phosphatase is discharged from the epithelium of the prostate in greater amounts than from the unstimulated gland. The observed decrease in alkaline-phosphatase in stimulated fluid

may be explained by a dilution of this enzyme, which is present in prostatic epithelium only in small amounts, by the greatly augmented amount of fluid secreted under the influence of pilocarpine.

The values obtained for ascorbic acid clearly show that it is concentrated by the seminal vesicle (table 3). In the semen of dogs, a species in which the seminal vesicle is lacking, ascorbic acid was found in low concentration, whereas in the semen of guinea pigs and man considerably higher values

TABLE 2

Concentration of acid and alkaline phosphatases in resting and stimulated semen of dogs

Units per 100 cc.

NUMBER	RESTING		PILOCARPINE STIMULATION	
	pH 5	pH 9	pH 5	pH 9
223	36.75	71.25	155	4.5
856	27.75	16.5	63.75	1.5
250	6	18	55.5	9
313	26.25	28.5	57.5	0.75
224	56.25	27.0	126.75	2.25

TABLE 3

Concentration of ascorbic acid in secretions of the male genital tract

Mgm. per 100 cc.

	GUINEA PIG			DOG		MAN				
	Ejaculate	Seminal vesicle fluid	Prostatic fluid	Prostatic fluid	Plasma	Ejaculate	Seminal vesicle fluid	Mixed seminal vesicle and prostatic fluid	Prostatic fluid	Spermatocele fluid
Mean	8.228	8.615	1.547	0.756	0.522	12.788	4.665	2.359	0.542	0.967
Standard deviation	0.707	0.790	0.308	0.076	0.039	0.828	0.847	0.345	0.013	0.185
Number of determinations	31	20	22	78	49	9	9	11	19	10

were obtained than was found for the plasma or whole blood of these species or the prostatic fluid of the dog. The data show that in man higher values of vitamin C obtain in the stimulated ejaculate than in resting fluid of the seminal vesicle, and still higher values than in resting prostatic secretion. Mixtures of prostatic and seminal vesicle secretions were intermediate in amount between seminal vesicle and prostatic fluids. Since acid-phosphatase is actively secreted by the stimulated prostate gland, it is reasonable to assume that stimulation of the seminal vesicle causes concen-

tration of ascorbic acid through active secretion of this weak acid by the epithelium of this structure.

In the guinea pig the concentration of ascorbic acid in semen obtained by electrical shock is slightly lower than that in the resting seminal vesicle fluid. Quantitative comparison of the weight of fluid found in the seminal vesicles and prostate of the guinea pig with the weight of ejaculate obtained from electrical shock, showed agreement in the weight of fluid obtained and indicated that electrical ejaculation consists chiefly of emptying of pre-formed contents of the glands rather than an active secretion. As further evidence, the concentration of ascorbic acid in semen following electrical ejaculation is only slightly lower than that in the seminal vesicle contents, a fact explainable by admixture of prostatic fluid. As in man, the ascorbic acid content of seminal vesicle fluid was higher than the prostatic fluid and is the principal source of the high concentration of this material in semen.

Huggins and Johnson (1933) showed that human semen contains large amounts of glucose, the chief source of which is the seminal vesicle. Since ascorbic acid has a similar 6-carbon carbohydrate structure, it appears that the seminal vesicle is well adapted for the secretion and concentration of these related molecular moieties.

Kiese and Hastings (1938) found that inhibition of alkaline phosphatase by ascorbic acid was slight and detectable only in concentrations above 0.01 M; and King and Delory (1936) concluded that no appreciable influence of ascorbic acid on phosphatase activity could be demonstrated.

CONCLUSIONS

Resting semen obtained without adventitious stimulation was compared with stimulated semen produced following the intravenous injection of pilocarpine; resting semen was about 0.6 pH unit more alkaline, contained slightly less CO_2, and considerably less chloride than stimulated semen. A reciprocal relationship was found in the concentrations of phosphatases of acid and alkaline pH optima in resting and stimulated semen. There was a decrease of alkaline phosphatase found in resting semen and an increase of acid phosphatase activity in the greatly augmented amount of fluid produced following pilocarpine stimulation. Intravenous injection of pilocarpine caused an active secretion by prostatic epithelium

Ascorbic acid was greatly increased in human and guinea-pig ejaculates as compared to plasma. The increased ascorbic acid largely derived from the seminal vesicle, which is able to concentrate this substance. In the dog, an animal without seminal vesicles, the ascorbic acid of semen was found at the plasma level. In guinea pigs ejaculation by electrical shock consists chiefly of the emptying of pre-formed fluids of the genital excretory tract rather than active secretion.

The seminal vesicle is the chief source of ascorbic acid in semen while the prostate gland contributes most of the acid phosphatase; the concentration increases during active secretion by these structures.

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A STUDY OF THE GASEOUS EXCHANGE BETWEEN THE CIRCULATORY SYSTEM AND THE LUNGS

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There have been many careful studies of the oxygen and carbon dioxide exchange between the lungs and the circulatory system. The literature reveals comparatively few quantitative measurements of the pulmonary gaseous exchange in the case of inert or inactive gases. In the present work radon[1] was chosen as the inert gas for several reasons. First, it is easy to detect and measure quantitatively by virtue of its radio-activity. Second, the amount that is needed is so minute (a few hundredths of a micro-gram) that it would defy chemical detection and hence probably could not introduce any extraneous physiological effect. Third, it is not normally present in the body. Consequently we avoid the complication of having to take account of an indefinite amount. Fourth, it is readily soluble in normal saline for injection into the blood stream.

PROCEDURE. The experiments were performed on dogs anesthetized with 25 mgm. of sodium pentobarbital per kilogram of weight. A known amount of radon dissolved in 9 cc. of normal saline was injected into the saphenous vein of the dog. The dog's expired air was collected in four rubber bags. The collection began just as the injection was started. The time required to shift the collection from one bag to another was negligible (about one second to operate the valves). The amount of radon in each bag was determined by placing it at a known distance from a Geiger-Muller counter. The results were plotted and the k computed. This brief outline of procedure will serve to correlate the following detailed description.

A radon solution was prepared by crushing the little glass capillary tubes containing the gas under a measured amount of normal saline, which was immediately drawn up into two syringes. One syringe containing 9 cc. was for injection and one containing 1 cc. was kept for a control, the procedure requiring from 3 to 6 minutes. The more quickly this was done the less the radon loss. Ordinarily, there was a total of 20 milli-curies distributed in about one-half dozen tubes.[2] Most injections were made

[1] Radium → Radon → RaA → RaB → RaC. RaC is the chief source of γ-rays.

[2] The radon was secured through the courtesy of the Steiner Clinic, Atlanta, Georgia.

into the saphenous vein. But since there was the possibility that a more direct route to the lungs would make a difference, a control experiment was performed in which the injection was made into a vein of the front leg in the region of the axilla. The computed values of the elimination constant, k, for these two sites of injection agreed within experimental error.

In order to avoid leaks about the dog's mouth, a sheet of thin rubber (dental dam) was wound around its nose and held in place with rubber bands. Then the mask was carefully slipped over the nose. The mask had a doughnut-like structure that could be inflated which made a soft but tight binding around the dog's nose. It was then connected to a glass

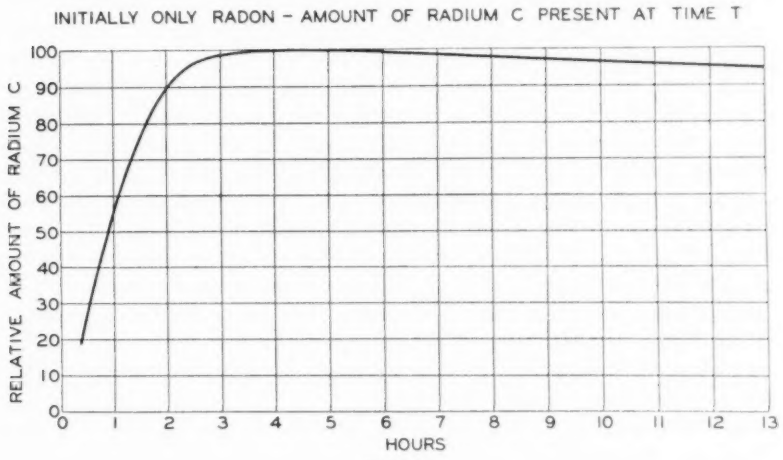


Fig. 1

tube with four side-arms, to each of which was fastened one of the rubber bags (football bladders). The volume of the system exclusive of the bags is difficult to estimate, because it is hard to judge the volume in the mask, which appeared to be between 75 and 150 cc. However, except for that of the mask, the dead air space was less than 50 cc. The total possible volume of the bags was about 12 liters, or approximately 3 liters per bag. On the average, the bags were blown up by the dogs to about 1400 cc. each. The collection time was varied from 45 seconds to 3 minutes per bag, the total collection time varying from 3 to 12 minutes.

The Neher-Harper (3) circuit was used to control the Geiger-Muller counter. The stabilized high voltage supply was patterned after that of Lifschutz (4). No scaling circuit was necessary, since for the quantities of radio-active material involved, the counting rates could be adjusted so as to remain within the resolution time of the mechanical counter by

varying the distance between the Geiger-Muller counter and the specimen. Since the γ-ray activity does not originate from the radon but from one of its disintegration products, radium C, it is necessary to wait about 4 hours after the collection of the radon for the accumulation of the maximum amount of radium C. The graph in figure 1 depicts the growth and decay of radium C when initially only radon is present. It is seen that between the 3rd and 7th hours is the optimum time to measure the γ-ray activity. It is during this interval that the γ-ray activity is a maximum and the change in activity is a minimum. Any radio-active deposit (i.e., Ra A, B, C) which might have been dissolved in the normal saline with the radon will have decayed to a negligible value by this time.

Because of the random nature of the disintegrations, the probable error in any given count is the square root of the number counted. Therefore, it is necessary to count 2500 counts for a 2 per cent error and 1,111 counts for a 3 per cent error. Hence, it is seen that the counting rate will determine the accuracy that it is feasible to obtain. The factor which limits the counting rate is the mechanical recorder, not the electronic control devices. The resolution time of the Cenco counter is about 0.01 second. The error introduced by the resolution time has been discussed by Locker and Weatherwax (5). It was found experimentally that, so long as the counting rate was less than 140 per minute, this error was less than 2 per cent. The distance of the specimen from the Geiger-Muller counter was adjusted so that the counting rate was about 120 per minute. Then, by use of the inverse square law, these various rates were reduced to a common basis at the 170 cm. distance. The least distance was 125 cm. and the greatest, 600 cm.

Calculation: The computations on the data of dog 5, May 1st, will illustrate the method.

SPECIMEN	COUNTS PER MIN. AT 170 CM.	$\log_e dQ/dt$	TOTAL COUNTS
1	234	5.45	234
2	99	4.59	333
3	56	4.02	389
4	28.5	3.34	417.5

Collection time 1 minute for each bag.

On the assumption that these data follow a single term exponential, we have the following relationships:

$$Q = Q_0(1 - e^{-kt}) \tag{1}$$

$$dQ/dt = Q_0 k e^{-kt} \tag{2}$$

Q is the quantity collected during the time, t. Q_0 is the initial quantity present. e is the logarithm base. k is the elimination constant. If the collection time for Q_b is twice that for Q_a, then it can be shown that:

$$k = 1/t_b \log_e Q_a^2/(Q_a - Q_b)^2 \tag{3}$$

Using $t_a = 2$ minutes and $t_b = 4$ minutes, we get:

$$k = 1/4 \log_e 333^2/(333 - 417)^2 = 0.69$$

However, the elimination constant, k, can be found in another way. The second equation shows that if the exponential law holds, then the graph of log dQ/dt versus the time, t, should be a straight line whose slope is k. The graph of these data is shown in figure 2. The slope of this line is seen to be 0.69 also. This excellent agreement is partly fortuitous, since there is some leeway in drawing the straight line. It is believed that the value of k obtained from the graph is better than the value obtained from the individual Q's since the line gives a weighted mean. The accuracy with which the points lie along a straight line indicates how well the exponential law applies to the data. The best and the worst graphs are shown in figure 3. The values of k computed from the graphs of the data obtained under various experimental conditions are shown in table 1. The time required for one-half of the gas to be eliminated is given by:

$$t_{1/2} = 1/k \log_e 2 \tag{4}$$

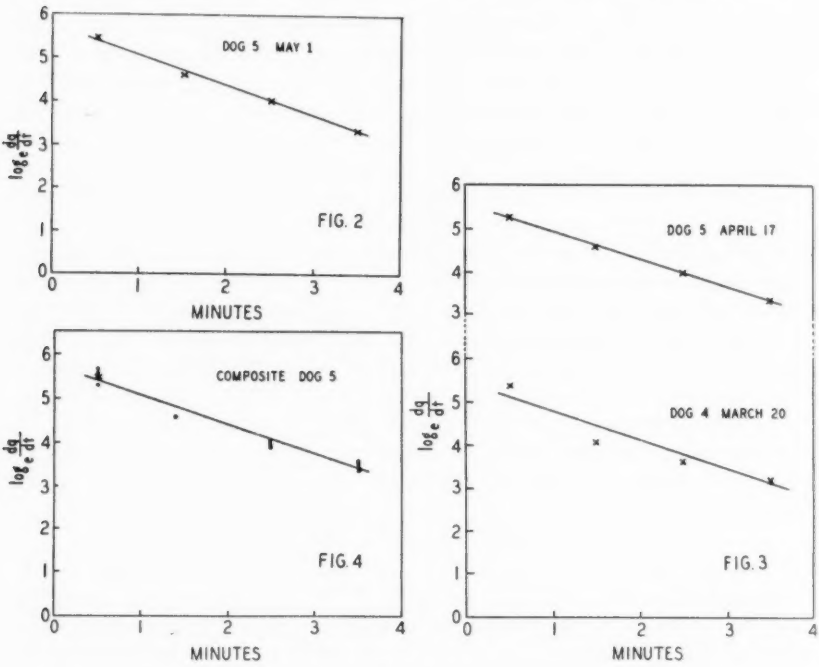
These half value times are also tabulated. The initial amount of gas Q_0 can be computed from the equation:

$$Q_0 = Q_a^2/(2Q_a - Q_b) \tag{5}$$

It will be recalled that at the time the radon tubes were broken under normal saline, two syringes were used, 9 cc. for injection and 1 cc. for control. The total amount of radon injected could be found by multiplying the counting rate of the 1 cc. amount by 9. This quantity is designated Q_0'. The discrepancy between Q_0 and Q_0' as revealed in table 1 will be explained later.

First, an effort was made to find out how well the data could be reproduced. Dog 2 was measured on three successive weeks and it is seen that the results vary by less than 3 per cent. Dog 3 was unique in that it had the lowest k of any measured, and the half value time was almost three times the others. We were unable to follow this up because the dog died the following day. The measurements on dog 5 were extended over a period of a month and under varying experimental conditions in an effort to find out what factors would influence k. The four sets of data are all plotted on a composite graph (fig. 4). The first data were obtained with

the normal procedure. In the second instance the dog's cardiac rate was slowed 63 per cent by the injection of 0.3 cc. of Veratone. In the third instance the radon was rapidly injected into a vein of the front leg instead of the hind leg as previously. In the fourth instance, the dog was subjected to a severe surgical operation. The experiment was performed immediately after the incision was closed. The cardiac output was undoubtedly affected by this operation (6), yet the results are the same within



Figs. 2–4

experimental error. This was a distinct surprise, since it was originally thought that the elimination of the gas would be dependent on the rate of circulation of the blood through the lungs. This indicates that even under adverse circumstances, the circulation in the lungs is more than adequate and that the elimination is limited by other factors. In obtaining the first data, dog 6 was under normal conditions for control. He was then subjected to a pneumothorax, in which 280 cc. of air were pumped into the pleural cavity, and the radon solution was injected immediately. Despite the pneumothorax, the total volume of air breathed during the 4 minutes was 5020 cc. In the previous experiment this dog exhaled a total of

4950 cc. Although the volume measurements are only reliable to within 50 cc., it is seen that the pneumothorax was essentially compensated by more labored breathing. Practically no variation is seen in the value of k obtained under these different conditions.

Finally, carbon dioxide was administered to cause hyper-ventilation. The volume of air was increased so drastically that the collection time had to be reduced from 1 minute to 45 seconds per bag. In 3 minutes 11,800 cc. were collected, which is to be compared with 3,650 cc. collected in the 3-minute interval previously. The administration of the carbon dioxide resulted in a 226 per cent increase in the volume breathed during the 3-minute interval. Inspection of the table shows that this caused an increase of 60 per cent in k.

TABLE 1

DOG NO.	DATE	Q_0'	Q_0	k	$t_{\frac{1}{2}}$ min.	CONDITION
1	12/ 7/39	490	285	0.540	1.29	Normal
2	2/ 8/40	828	420	0.696	1.00	Normal
2	2/14/40	900	427	0.696	1.00	Normal
2	2/21/40	927	455	0.680	1.02	Normal
3	3/ 6/40	1260	420	0.240	2.90	Normal
4	3/20/40	592	346	0.670	1.04	Normal
5	4/17/40	600	390	0.680	1.02	Normal
5	5/ 1/40		447	0.690	1.01	Heart slowed 63 per cent
5	5/ 8/40	1035	620	0.660	1.05	Rapid front leg injection
5	5/15/40		480	0.660	1.05	Severe operation
6	5/22/40	1070	465	0.570	1.22	Normal
6	5/29/40	540	253	0.575	1.22	Pneumothorax
6	6/ 5/40	810	760	0.920	0.75	Hyper-ventilation

It must be remembered that all of the discussion of our data so far has been on the assumption of a single exponential. This assumption is justifiably applied to our data only during the short time interval. The discrepancy between Q_0 and Q_0' is explained by assuming that only a portion of the original Q_0' remains in the active pulmonary circulation as Q_0. The remainder goes either into regions of poor circulation or into tissue and hence has a much smaller k and therefore would not be measured in the short time intervals used in this experiment.

The absorption and elimination of nitrogen has been studied by Shaw, Behnke, Messer, Thomson and Motley (1) and by Behnke, Thomson and Shaw (2). The elimination of nitrogen was determined by allowing the person or animal to breathe an atmosphere of pure oxygen in a closed circuit. Samples of the atmosphere were taken for analysis at different times. Even at the end of a run the oxygen concentration was never less

than 96 per cent. Therefore, it required extreme accuracy in the gas analysis to measure quantitatively the nitrogen eliminated. This work shows that nitrogen is eliminated in a way that can be described by a sum of exponential functions.

$$Q = \sum_i {}_iQ_0(1 - e^{-k_i t})$$

where Q is the total amount collected during the time interval, t. ${}_iQ_0$ is the inital amount present in the i state (i.e., in water, fat, etc.). The k_i is the elimination constant for the i state and determines the quantity eliminated per unit of time.

There is a further complication in the case of elimination of a gas from the *body* through the lungs; (since the blood stream is the intermediary between tissues and lungs), it is necessary to consider the elimination constant k for the gas entering and the k for the gas leaving the blood. If the k for the gas leaving the blood is considerably larger than the k for that entering, then after the first initial gas present in the blood is eliminated, the remaining process will be limited by the smaller k of the entering gas. We believe that our experimental work gives a direct measure of the k for the gas leaving the blood stream. On the other hand, the work of Shaw et al. (1) and Behnke et al. (2) on nitrogen elimination establishes the entering k. Since, according to the information on solubility in these references, there are only about 30 cc. of nitrogen in the blood at atmospheric pressure, and the quantity eliminated during the first 5 or 10 minutes was not collected, the k for this amount was not detected. Behnke's (2) short time values for k for nitrogen range from 0.0785 to 0.099. Our values of k for radon are about six times as high as these values. It is not strictly proper to compare data obtained on dogs and data obtained on man. But, in view of the essential agreement between the data on dogs and patients in the two references cited, this difference of six-fold in k appears to be real. In fact, we believe that it solves the paradox encountered by Shaw et al. (1) which forced them to conclude that a gas can exist in vivo in a peculiar state of super-saturation not encountered in vitro. For, if we assume that our k of about 0.66 is the one applicable to a gas leaving and the value of 0.089 for a gas entering the blood stream from the fluids in the tissues, we have a situation closely analogous to a radio-active disintegration series, provided we assume that only the one entering k is significant. This means that we are ignoring the k of 0.0085, which Behnke (2) attributes to the gas in the fat. This is allowable over a short interval of time but, for example, it is over a short interval of time that the problem of too much gas in the body during decompression is acute. The mathematics involved are rather intricate and the boundary conditions will determine the type of solution obtained. Since we do not have adequate information about the boundary conditions, it is not profitable

to present a mathematical analysis. However, we can point out a certain generalization. In a series of different states characterized by definite $\frac{1}{2}$ value periods, the shorter the $\frac{1}{2}$ value period, the smaller the quantity in that state. Therefore, the greater the k of the gas in the blood stream in comparison to the k in the tissues, the smaller is the amount of gas that will be in the blood at any given instant.

This may explain the findings of Behnke, Shaw, Messer, Thomson and Motley (7), that the administration of pure oxygen instead of air to persons and dogs undergoing decompression greatly improves their condition, because the high value of k is obtained for a given gas only when the concentration of that gas is zero in the inspired breath. Nitrogen is the chief source of the emboli, hence no nitrogen should be inhaled. Our work indicates that the administration of small amounts of carbon dioxide to produce hyper-ventilation should be beneficial, since it increases the value of k.

CONCLUSION

The elimination of radon directly from the blood stream by way of the lungs has been shown to obey an exponential law. The value of the elimination constant, k, has been determined under different conditions. This constant, k, was not influenced by variations of 1, pulse rate; 2, cardiac output, or by 3, pneumothorax. Hyper-ventilation caused a drastic change in k. The administration of carbon dioxide, by respiratory stimulation, will speed up the process of pulmonary elimination of gas from the blood and will tend to prevent the accumulation of excess gas in the circulatory system from the tissues. There is no need to postulate the existence of a peculiar state of supersaturation in vivo, as has been done by Shaw et al. (1).

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EXCITATION OF INTRASPINAL MAMMALIAN AXONS BY NERVE IMPULSES IN ADJACENT AXONS

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Well-known experiments made with the rheoscopic nerve-muscle preparation reveal that the action currents of muscles can stimulate nerve fibers. It is less generally recognized that under certain conditions axons can be effectively stimulated by the activity of adjacent axons (Hering, 1882). Hering worked with the nerves of winter frogs. In one version of his experiment he prepared the peroneal and tibial nerves for stimulation, and observed the muscles innervated by other branches of the sciatic (fig. 1 *a*). For a few minutes after making a cut across the sciatic plexus, the delivery of a weak induction shock to the tibial and peroneal nerves was followed by a powerful contraction of the adductor muscles of the thigh. Hering made careful controls, which demonstrated that the motor axons of the adductor muscles were not excited by escape of the electrical stimulus applied to the primary (conditioning) axons, nor by spread of electrotonic changes. He was, therefore, led to believe that the impulses in the primary axons directly stimulated the secondary (tested) fibers.

Hering emphasized that the following conditions contributed to the success of his experiment: 1, the preparations were very excitable; 2, the two groups of fibers, conditioning and tested, converged and came to lie in intimate topographical association; 3, the common bundle containing the two groups of axons was freshly cut across. When these conditions are fulfilled, it is not difficult to perform a modification of Hering's experiment with frog nerves and to confirm his results (cf. also von Uexküll (1894) and Kwassow and Naumenko (1936)). Recently, several papers have reported the stimulation of hyperexcitable invertebrate axons by the activity of adjacent fibers (Jasper and Monnier, 1938; Katz and Schmitt, 1940; Arvanitaki, 1940a, b, c). In addition it has been shown that subthreshold alterations in the excitability of tested axons can be detected in experiments in which propagated secondary impulses are not initiated by impulses in neighboring conditioning axons (Otani, 1937; Katz and Schmitt; Blair and Erlanger, 1940).[1]

[1] Since the present paper was sent for publication, two additional papers on cross excitation between medullated axons have appeared (Feng and Li, Proc. Soc. Exper. Biol. and Med. **45**: 870, 1940; Rosenblueth, This Journal **132**: 119, 1941).

The intraspinal ascending branches of the afferent neurons of the mammalian spinal cord offer a preparation in which the conditions necessary for an experiment analogous to that of Hering are easily attained (fig. 1 *b*), since the ascending branches of adjacent dorsal root fibers lie in the same portion of the dorsal column. If one of two adjacent dorsal roots be stimulated and a fresh transection be made of the dorsal column cephalad to the root level, impulses passing up the column in the axons belonging to the stimulated root will excite in the column the axons belonging to the other root, and the impulses so set up may be recorded as a centrifugal volley in the latter. The central latency for the centrifugal impulses is so brief that the stimulation of the secondary axons can be effected only by processes contemporaneous with the spike potential in the primary axons.

Barron (1940) suggests that direct stimulation of the intraspinal branches of dorsal root fibers by impulses in adjacent axons may account for the centrifugal impulses of brief central latency which were observed in the dorsal roots by Matthews and himself (1935). In our experience, secondary impulses of very brief central latency have been observed only after section of the dorsal column.

Methods. The experiments were made on cats under Dial narcosis (Ciba, 0.6 cc./kgm.). In each experiment a laminectomy was performed and the dura opened. Usually two groups of lumbo-sacral dorsal rootlets were cut intradurally and prepared for stimulating (primary or conditioning group, C, fig. 1 *b*), and recording (secondary or tested group, T). In several experiments in which all dorsal roots were left intact, the sciatic nerve was stimulated just above the knee and records were taken from the sural nerve in the popliteal space. In some cases the reflex discharges evoked in the ventral roots by stimulation of the conditioning dorsal rootlets were examined. Action currents of the dorsal column axons were recorded, and these axons were also directly stimulated, through small Ag-AgCl electrodes placed upon the dorsum of the cord (g_1 and g_2, fig. 1 *b*). The preparations were covered with paraffin oil to a depth of about one centimeter, in order to help maintain the cord and its roots in good condition for long periods of time. The customary differential amplifier and stimulating apparatus were used.

Results. Stimulation of a group of dorsal rootlets (*C*, fig. 1 *b*) with a single shock maximal for alpha fibers produced in adjacent rootlets, *T*, the dorsal root reflex (fig. 2 *a*). As Toennies (1938) and Hursh (1940) have shown, the central latency for this discharge varies between 2.1 and 3.5 msec., depending upon the temperature of the preparation. There has been no indication that centrifugal impulses emerged from *uninjured* cords after shorter latencies. After section of the dorsal column cephalad to the point of entry of the conditioning and tested dorsal root fibers,

however, a striking change appeared in the oscillograms. A conspicuous deflection, considerably preceding that caused by the dorsal root reflex, occurred (fig. 2 *b*, *c* and *d*). Controls demonstrated that this deflection was due to impulses which were conducted centrifugally in the secondary fibers (fig. 3). The response was diphasic when led from two electrodes on the live tested fibers (fig. 3 *a*), and monophasic when the tested rootlets were crushed under the distal electrode (fig. 3 *b*). Further, the latency increased as the proximal lead was moved distally on the tested axons, and its size did not decrease rapidly, as would have occurred if the response were being led electrotonically from the cord. The possibility that the secondary fibers were stimulated intracentrally by spread, either of the stimulating current or of electrotonic changes in the conditioning axons, was tested and excluded by reversing the stimulating leads (fig. 3 *c*); the early centrifugal impulses were then still present and bore the same temporal relation to a small deflection (marked with arrow), which indicated the arrival of the conditioning volley at the cord. No significant current flowed from the stimulating transformer through the preparation to ground, for there was no response when one of the stimulating leads was disconnected from the preparation (fig. 3 *d*).

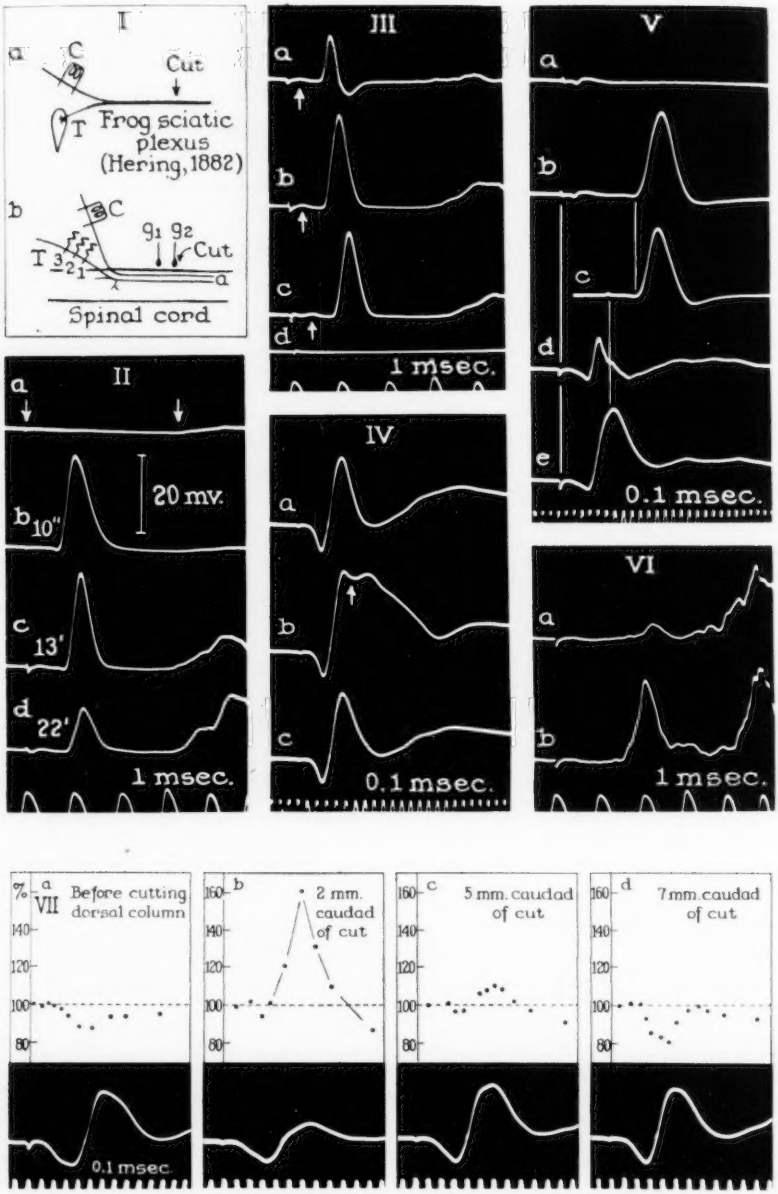
Additional experimental support exists for the contention that the early volley of centrifugal impulses is not dependent upon the proximity of the stimulating and recording electrodes to the cord or to the region of transection of the ascending axons. 1. The early centrifugal volley has appeared when the level of transection was as much as 40 mm. cephalad to the level of root entry. Its latency was then greater than when the cord was cut nearer the level of root entry. The increase in latency could be accounted for by the greater distances of conduction in the primary and secondary axons. 2. In four experiments the sciatic nerve was stimulated just above the knee and records were taken from the sural nerve, which contains only sensory fibers. When the spinal cord was intact, only the impulses of the dorsal root reflex appeared in the sural nerve. After transection of the dorsal column at the level of the 6th or 7th lumbar segment, the electrogram of the sural nerve showed, in addition to the dorsal root reflex, an earlier deflection comparable to that of figure 2 *c*. It must be concluded that the tested axons were excited intracentrally as a result of the activity of adjacent conditioning axons.

As is shown by the records of figure 2, the number of tested fibers excited by a given conditioning volley decreased progressively with the time that had elapsed after the transection of the dorsal column. With some favorable, cool preparations, such as that from which the records of figure 2 are taken, the volley in the secondary fibers initially amounted to as much as 25 millivolts and involved more than one-half of the alpha fibers of the tested rootlets; and centrifugal impulses could still be detected as much as

half an hour later. In warm animals, as would be expected, the restitution of the cut axons proceeded more rapidly and the response usually disappeared within a few minutes or even seconds after the transection. Thus, although the response did occur when the dorsal columns were at temperatures normal for cats, it was more favorably examined in cooler preparations.

The temporal relation between the arrival of the conditioning impulses at the region near the cut and the initiation of impulses in the adjacent tested axons is of interest. Figures 5 *a* and *b* reveal that a conditioning centripetal volley which entered the cord in the fibers of a group of dorsal rootlets produced a centrifugal volley in adjacent rootlets after, but not before, transection of the dorsal column about 15 mm. cephalad to the level of root entry. Records *d* and *c* show the potential changes recorded between an electrode (g_2, fig. 1 *b*) on the dorsal column at the level of transection and a second electrode (g_1) placed 2.5 mm. caudad to g_2. Record *d* was made just before transection of the dorsal column and record *c* about 20 minutes later. Record *c* shows a volley initiated in the tested dorsal root fibers by a cathodal shock applied through electrode g_1. The shock-response interval included a short utilization time, estimated to have been about 0.1 msec., and the conduction time from the region caudad of the cut to the recording electrodes. Examination of the oscillograms with reference to the simultaneous ordinates which have been drawn makes it clear that the tested fibers were excited at the time when the spike negativity of the conditioning axons existed at the region caudad to the cut. The results of a number of experiments have established that the centrifugal discharge arrived at the recording electrodes only 0.1 to 0.3 msec. later than it would have if the conditioning volley had travelled in an uninterrupted fiber path from the stimulating cathode cephalad to the region of the cut and back to the recording electrodes.

The fact that secondary axons were excited during the period of negativity due to the spike potential of the conditioning impulses is likewise demonstrated by an examination of the records of figure 4. The potential changes attributable to a conditioning volley were recorded from the electrode g_1 on the dorsal column and an indifferently placed electrode. Record *a* was taken before section of the dorsal column. Record *b* was obtained immediately after transection of the column about 2.5 mm. cephalad to g_1, and record *c* about 20 minutes later. Records *a* and *c* show an initial deflection which is referable to the afferent volley; this is followed by the first part of the negative cord potential (Gasser and Graham, 1933). In record *b* an arrow marks an additional deflection which must be interpreted as caused by impulses that were set up in secondary fibers by impulses of the conditioning volley. In confirmation of the conclusion derived from the records of figure 5, the tested fibers were stimulated at approximately


I
a
C
Cut
T
Frog sciatic plexus (Hering, 1882)
b
C
g1 g2
Cut
T 3 2 1
a
Spinal cord
II
a
20 mv.
b 10"
c 13'
d 22'
1 msec.
III
a
b
c
d
1 msec.
IV
a
b
c
0.1 msec.
V
a
b
c
d
e
0.1 msec.
VI
a
b
1 msec.
% a VII Before cutting dorsal column
160
140
120
100
80
0.1 msec.
b 2 mm. caudad of cut
c 5 mm. caudad of cut
d 7 mm. caudad of cut

Figs. 1–7

the time that the negativity due to the primary impulses was maximal a few millimeters caudad to the cut.

The negative cord potential is a sign of the activity of the spinal interneurons which are stimulated by a primary afferent volley (Gasser and Graham, 1933). Therefore, the activity of postsynaptic elements in the cord cannot be responsible for the excitation of the tested axons, because the secondary impulses are set up before the beginning of the negative cord potential (figs. 4 and 5).

Subthreshold changes in the excitability of the tested axons could be detected even after the conditioning volley had ceased to initiate secondary impulses. These subliminal excitability changes were measured as follows: A cathodal shock applied through electrode g_1 (fig. 1 *b*) stimulated the ascending branches of some of the dorsal root fibers labelled *T* in figure 1 *b*.

Fig. 1 *a*. Diagram of Hering's experiment. The fibers of the peroneo-tibial nerve (*C*) intermingle in the sciatic plexus with the axons that supply the adductor muscles of the thigh (*T*). For a short time after cutting across the sciatic plexus, stimulation of the peroneo-tibial nerve at the knee initiated contractions of the adductor muscles. *b*. Diagram for the present experiments. The ascending branches (*a*) of adjacent dorsal rootlets (*C*, *T*) lie in close topographical association in the dorsal columns of the spinal cord. For a period of time after transection of the dorsal columns at g_2, a centrifugal volley, which entered the cord over the fibers of one group of dorsal rootlets (*C*), served to initiate a volley of centrifugal impulses in the fibers of adjacent dorsal rootlets (*T*). Activity in the dorsal columns was recorded *via* electrodes (g_1, g_2) placed on the dorsum of the cord. g_2 was located at the level of transection, g_1 about 2.5 mm. caudad of g_2.

Fig. 2. The stimulation of dorsal column axons by impulses in adjacent axons. Conditioning dorsal rootlets: first sacral (S_1) and cephalic two-thirds of the 7th lumbar (L_7). Tested rootlets: caudal one-third L_7. Record *a*, before transection of the ipsilateral dorsal column 5 mm. cephalad of L_7. Record *b*, 10 seconds after transection; *c*, 13 minutes; *d*, 22 minutes. The amplification for records *a* and *b* is indicated by the voltage calibration on the figure; record *c*, 5 ×; record *d*, 25 × the amplification of *b*. The first arrow marks the escape of the conditioning shock; the second arrow indicates the onset of the dorsal root reflex discharge. Rectal temperature, 36.6°. Time as indicated.

Fig. 3. Same experiment as figure 2. The tested axons were alive under electrodes *1* and *2*, killed under *3* (fig. 1 *b*). Record *a*, the diphasic response recorded from electrodes *1* and *2*. Record *b*, monophasic response recorded from *1* and *3*. Record *c*, as *b*, but stimulating leads reversed so that the cathode was in the distal rather than in the usual proximal position. The arrow marks a small deflection which is referable to the arrival of the conditioning volley at the cord. Record *d*, one stimulating lead disconnected from the preparation. Time as indicated.

Fig. 4. Impulses in the conditioning and tested fibers, as recorded from the dorsal column. The records are from electrode g_1 (fig. 1 *b*) placed about 2.5 mm. caudad of the level of transection and an indifferent electrode. Stimulated (*C*) dorsal rootlets: cephalic one-half L_7. Record *a* was taken before the dorsal column was transected; record *b* immediately after transection; record *c*, about 20 minutes later. The deflection marked by the arrow in record *b* is due to impulses initiated in second-

The impulses travelled caudally and emerged as a submaximal centrifugal volley in the fibers T. The changes in the size of this volley, which were induced by a preceding conditioning volley ascending from rootlets C, served as measures of the excitability changes of the tested axons.

The observed excitability changes are in complete accord with the changes observed in frog nerves by Blair and Erlanger (1940). So long as the dorsal column remained intact and uninjured, the excitability of the tested axons was decreased during the period of spike negativity in the adjacent conditioning axons (fig. 7 *a*). Transection of the dorsal column produced a complete change in the excitability curves determined at regions close to the cut. The data of figures 7 *b*, *c* and *d* were obtained several minutes after the cut had been made—after the conditioning volley had ceased to initiate secondary impulses. The oscillogram and curve of figure 7 *b* were obtained with g_1 at a point 2 mm. caudad to the cut. A large *increase* of the excitability of the tested axons occurred during the period of relative negativity which was produced by the conditioning impulses. Five millimeters caudad to the cut the increase of excitability

ary axons by the impulses in the ascending branches of the conditioning L_7 dorsal root fibers. This deflection and the activity it represented had largely disappeared several minutes later (record *c*). Negativity at g_1 is recorded as an upward deflection. Rectal temperature, 36°. Time as indicated.

Fig. 5. Conditioning dorsal roots: S_1 and cephalic two-thirds L_7. Tested dorsal rootlets: caudal one-third L_7. Records *a*, *b*, and *c* are from the tested dorsal rootlets (T, fig. 1 *b*). Records *d* and *e* are from the dorsal column (bipolar leads from g_2 at the level of the section and g_1 2.5 mm. caudad to it). The stimulus for records *a*, *b*, *d* and *e* was a shock applied to the C rootlets; for record *c* a cathodal shock was delivered to the dorsal column at electrode g_1. Records *a*, *c* and *d* were obtained before transection of the dorsal column 15 mm. cephalad of L_7; records *b* and *c* after the section had been made. Rectal temperature, 36.9°. Time as indicated.

Fig. 6. Effect of dorsal column section on motor discharge. The 7th lumbar dorsal root was stimulated and records were taken from the corresponding ventral root axons. Record *a* before, and record *b* 30 seconds after, a transection 15 mm. above L_7. The transection involved little, if any, of the cord other than the dorsal column. Cool preparation. Time as indicated.

Fig. 7. Subthreshold excitability changes induced in tested axons by a conditioning volley in adjacent axons. The testing stimulus was a submaximal cathodal shock applied at g_1 (fig. 1 *b*). The tested response was recorded through the electrodes on one-half of the L_7 dorsal rootlets (T). The conditioning activity was a volley in the other L_7 dorsal root fibers (C). The oscillograms are records of the potential changes which were set up at g_1 by the conditioning volley in isolation. Ordinates of graphs: $\frac{\text{height of conditioned tested response}}{\text{height of unconditioned tested response}} \times 100$. Abscissae: interval at which the testing shock followed the conditioning stimulus. *a*, before transection of the dorsal column; g_1 was about 15 mm. cephalad of the 7th lumbar segment. Similar curves were obtained for other axial positions of g_1. *b*, *c* and *d*, after transection of the dorsal column about 16 mm. cephalad of the 7th lumbar segment. The distance of g_1 caudad of the cut is indicated on the figure for each curve.

was much less (fig. 7 *c*); and at 7 mm. (fig. 7 *d*) the excitability curve approached that which obtained before injury.

Thus the greatest increase in the excitability of the tested axons occurred 1, at regions a short distance (2± mm.) caudad to the cut; and 2, at the time when the negativity due to the conditioning impulses was greatest at this locus. These findings are in accord with the fact that, when the tested axons were effectively stimulated by the conditioning volley, the secondary impulses arose at approximately the time the conditioning volley produced the greatest relative negativity a short distance caudad to the transection (figs. 4 and 5).

Figure 6 shows that reflex discharges, evoked in a lumbar ventral root by stimulation of a group of dorsal rootlets, were greatly augmented immediately after section of the ipsilateral dorsal column. A few minutes after the transection had been made, the reflex had reverted to approximately its original size. These findings were not unexpected, because for a short while after transection of the dorsal column the reflexogenic action of an afferent volley must be supplemented by the effects of impulses in secondarily excited afferent neurons. Thus the direct excitation of dorsal column axons by impulses in adjacent axons is one of the factors responsible for the immediate increase in spinal reflexes which is induced by cord section (Sherrington and Sowton, 1915; Forbes, Cobb and Cattell, 1923). It is clear that additional factors must be involved when complete transection of the cord induces an increase that persists for prolonged periods of time.

Discussion. The present experiments with intraspinal mammalian axons confirm Hering's (1882) original findings for frog nerves. The controls which have been made in both instances demonstrate that the excitation of the tested axons is not to be explained as an artefact caused by stimulus escape or by the spread of electrotonic changes from the stimulating electrodes to the tested axons (cf. the "paradoxical contraction" of duBois-Reymond, 1849). Hering presumed that the tested axons were stimulated by the "negative variation" of the primary axons. The present experiments prove the likelihood of this supposition, because they show that secondary impulses arise approximately at the time the relative negativity due to the conditioning impulses attains its maximal value near the transection (figs. 4 and 5). This point deserves emphasis because a different result has been obtained in experiments with unmyelinated invertebrate axons (Jasper and Monnier, 1938; Arvanitaki, 1940a, b, c).

Subthreshold changes in the excitability of medullated frog axons are induced by impulses in adjacent fibers (Blair and Erlanger, 1940). Blair and Erlanger find that, near the region of a cut or an injury, the excitability of tested fibers is increased by the arrival of conditioning impulses in adjacent axons. Figure 7 reveals that comparable changes occur in the

tested intraspinal axons. Hering's experiments and the present results show that this increased excitability sometimes attains threshold, and impulses are initiated.

The direct excitation of tested axons by the action currents of adjacent axons has been observed only after section of or injury to the common bundle of conditioning and tested axons. There are two apparent reasons why the proximity of a region of fresh injury might facilitate the stimulation of the tested axons. First, the external electric field which the conditioning impulses produce as they approach the region of the cut is altered, so that it may be a more effective stimulus (fig. 5 *d* and *e*). Second, the excitability of regions of the tested axons adjacent to the cut is temporarily increased after the production of the injury (Hering).

Since under certain circumstances the action current of axons can effectively stimulate other, anatomically independent axons, it is obvious that a possible anatomical discontinuity at synapses offers no *a priori* reason for assuming that the action currents of pre-synaptic fibers and endings could not excite post-synaptic neurons.

It seems likely that the excitability of neurons in the central nervous system may depend not only upon the effects produced by the arrival of impulses at synapses, but also by the environmental changes produced by the activity of neighboring neurons. As Grundfest (1940) has suggested, the excitability changes produced in axons by the activity of adjacent axons are, therefore, of interest as examples of the effects that may occur in the more complex systems.

SUMMARY

The dorsal column of the spinal cord contains the ascending branches of sensory fibers which enter the cord over the ipsilateral dorsal roots. For a period of time after transection of the dorsal column, at a level cephalad to the entry of a stimulated dorsal root, impulses in the ascending branches of the active fibers directly excite adjacent axons. The impulses in the secondary axons then travel antidromically (caudally) and emerge as a centrifugal discharge in dorsal root fibers adjacent to those which carried the centripetal volley. The secondary impulses are initiated by processes contemporaneous with the arrival of primary impulses at the region caudad to the cut, and before post-synaptic spinal neurons become active.

Subthreshold increases in the excitability of tested dorsal column axons are produced by a primary volley which does not actually initiate secondary impulses. The increase in excitability is greatest a few millimeters caudad to a cut. At this locus the maximal excitability coincides with the time at which the conditioning impulses produce the greatest relative negativity. Before section of the dorsal column, the excitability of tested axons is *decreased* by impulses conducted in adjacent axons.

Transection of the dorsal column produces an immediate increase in the size of the motor discharges that are evoked by dorsal root volleys.

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EFFECTS OF ADRENALIN AND ACETYLCHOLINE ON ISOLATED IRIS MUSCLE, IN RELATION TO PUPILLARY REGULATION[1]

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In attempts to answer a number of questions relative to the pupillary changes which occur in animals exposed to oxygen at high barometric pressure, experiments were performed on isolated iris muscle. The present communication is a consideration of the effects of adrenalin and acetylcholine, the administration of which substances was incidental to the study of the action of oxygen at high pressures on iris muscle.

The iris tissue used was taken from recently killed ox, from dogs previously anesthetized with morphine and urethane, and from rabbits killed by postcephalic blow by hand. The sphincter and radial muscles were studied separately and were mounted by the suspension method in various physiological solutions. In most of these experiments Tyrode solution was employed but in a few, phosphate Tyrode (Garry, 1928) and Ringer's solutions were used to check on the results obtained in Tyrode solution.

Throughout each experiment the solution employed was maintained at a constant temperature of 37.5°C. Provision was also made for bubbling the bath at a uniform rate with oxygen, nitrogen, or a mixture of these gases as might be desired. The gas used was previously saturated with water vapour to prevent undue change of salt concentration of the bath which otherwise might occur as a result of evaporation. Changes in tonus or contractions were recorded either photographically by an isotonic optical lever, or on smoked paper by a very delicate mechanical lever. All of our tissue preparations proved so rugged in their reactions as to make the refinement of photographic recording quite superfluous; it was therefore dispensed with in our later experiments. Although in preliminary tests it was found that a light waxed silk thread was unaffected by the bath solutions or the chemicals used in our experiments and therefore should not introduce unsuspected artefacts, most of our tissue preparations were suspended by a very fine enamelled wire and thread so that the latter never came into contact with the bath solutions or the chemicals added thereto in the various procedures.

[1] These experiments were supported by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

IRIS SPHINCTER MUSCLE. The iris sphincter was prepared by making an incision concentric with the pupillary margin so as to free a continuous ring of tissue about 3 mm. wide and suspending it in the bath.

Adrenalin. Adrenalin added to the bath in various dosages as to give final concentrations of from 1:10,000,000 to 1:100,000 produced a decrease in tonus of the sphincter muscle as shown in figure 1. This sphincter relaxing action of adrenalin, which was found to occur in isolated iris of ox, dog and rabbit, is in accord with the experimental findings of Poos (1927) and constitutes further confirmatory evidence that the sphincter fibers of the iris are profoundly influenced by sympatho-mimetic sub-

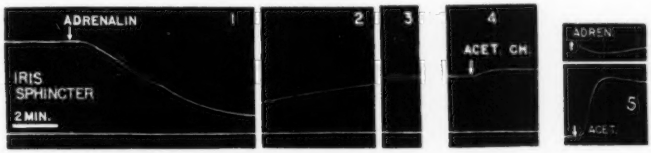

Fig. 1. Part 1. Drop in tonus of ox iris sphincter muscle elicited by adrenalin (final concentration 1:1,000,000). Parts 2 and 3. Recovery. Seven minutes elapsed between the records shown.

Part 4. The effect of acetylcholine (1:50,000) on ox iris sphincter muscle.

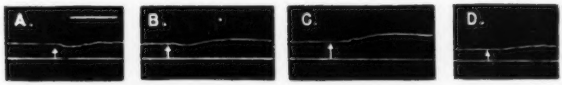

Fig. 2. Exceptional contracting effect of adrenalin on iris sphincter (dog). In the first administration, A, (final concentration 1:2,500,000) the response was predominantly one of relaxation; in a second similar administration, B, an initial relaxation is followed by contraction. In subsequent administrations, C and D, the response is one of contraction only. The bar in A represents 2 minutes.

stances liberated *in vivo* by the sympathetic nerve endings. There are, however, what may be very significant exceptions to this predominant relaxing effect of sympatho-mimetic substances on iris sphincter. This was observed in several experiments on isolated iris of the dog and cow, in which adrenalin administration, after eliciting a slight initial relaxation, caused a contraction as shown in figure 2.

Acetylcholine. Acetylcholine, as might be expected, elicited contractions in the isolated sphincter muscle preparations, as shown in figure 1 (part 4). However, in view of the fact that the iris sphincter muscle is capable of contracting 87 per cent of its relaxed length (Adler, 1933) and that the dose of acetylcholine administered was relatively strong (final concentration 1:40,000) the response of our relaxed muscle preparations to this parasympatho-mimetic substance was, with the one exception shown in

figure 1, part 5, surprisingly small. Administrations of acetylcholine in amounts less than that necessary to give a final bath concentration of 1:60,000 were almost invariably without any apparent effect. Those preparations which had been kept in oxygenated Tyrode at low temperature for 24 hours and which should, therefore, have had an increased sensitivity (Cannon and Rosenblueth, 1937) were likewise unresponsive to acetylcholine except in large doses. The use of similarly large doses (final concentration 1:66,666) by other investigators (Heath and Geiter, 1939) to elicit a good contraction in this muscle, supports the contention that isolated iris sphincter is possessed of a low sensitivity to this parasympatho-mimetic substance. Such low sensitivity contrasted with the relatively high sensitivity of the sphincter to inhibitory sympatho-mimetic substance is strongly indicative that the major control of the sphincter muscle is vested in its sympathetic innervation.

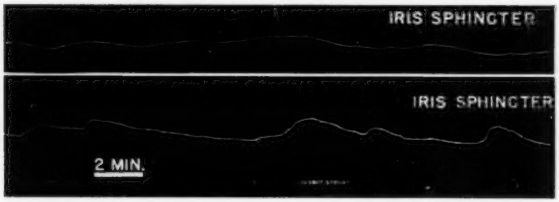

Fig. 3. Inherent rhythmicity of ox iris sphincter. Two different preparations

Spontaneous rhythmicity of iris sphincter muscle. Spontaneous rhythmical contractions of such magnitude as to be easily recorded on smoked paper frequently occurred in our iris sphincter preparations. This rhythmicity, with few exceptions, was most prominent during those periods in which the muscle was in a relaxed or partially relaxed state—such as that obtaining during the cessation of oxygen bubbling (fig. 3). Similar, though very much less prominent, spontaneous rhythmical contractions occurred in the preparations during short exposures to bubbling with nitrogen. The length of the spontaneous contraction waves varied but for the most part was of from four to one-half minutes' duration from crest to crest.

The pronounced inherent rhythmicity of the iris sphincter muscle is of more than passing interest in view of its importance as a very probable contributor to the occurrence of pupillary play. The play of the pupil, frequently referred to as hippus, has been generally interpreted as having its site of origin within the central nervous system; but the results of our experiments suggest that under certain conditions it could as well be due to an interruption of those extrinsic nerve impulses which normally exert a controlling influence over the inherent rhythmicity of the muscle. In other words hippus may result from *releasing* the inherent muscular rhyth-

micity from its superimposed extrinsic nervous control, rather than from some peculiar nerve centre rhythmicity mediated to the iris by its nerve connections.

RADIAL IRIS MUSCLE. Isolation of the radial muscle was accomplished by first placing a fine, three tined metal hook through the iris at the pupillary margin and then carefully freeing a narrow sector by two incisions carried peripherally from the pupillary margin through the iris and sclera to the base of the iris. The preparation so isolated was suspended in the physiological solution from a very light straw lever by thread and the three tined hook. The length of the metal hook made it unnecessary to have any part of the thread submerged in the fluid.

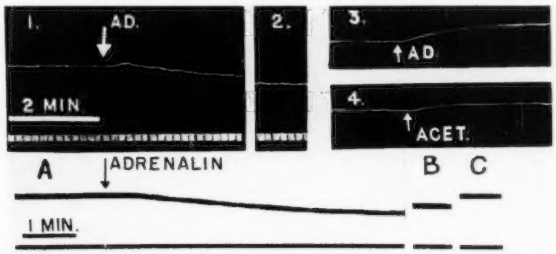

Fig. 4. A, B, C. Kymograph record. Part 1. Predominant decrease in tonus of radial muscle of ox iris as the result of adrenalin administration (final concentration 1:1,000,000). Part 2. Tonus of the same preparation four minutes later.

Parts 3 and 4. Rabbit iris radial muscle preparation. Part 3. Contraction elicited by adrenalin (1:4,000,000). Part 4. Contraction of radial muscle elicited by acetylcholine (1:1,000,000). Drum speed same as in part 1.

Photographic record: Parts A, B and C. Effect of adrenalin on ox iris, and recovery.

Adrenalin and radial muscle. The radial muscle preparations were much less responsive to experimental procedures than were the sphincter preparations. Adrenalin added to the bath of radial muscle of the rabbit iris, in amount sufficient to give a final concentration of from 1:5,000,000 to 1:500,000 elicited a contraction of the muscle (fig. 4, part 3). The radial muscle of the dog was affected in similar manner. The response of the radial iris muscle of the ox to adrenalin, however, was almost invariably a relaxation as is shown in figure 4, parts 1 and 2, and A, B, C. This inhibitory action of adrenalin on ox iris radial muscle was not limited to any one of the three physiological solutions employed as the bath. pH tests of the bath in several experiments likewise offered no clue as to the cause for this unexpected relaxation. It would appear then that the response of isolated radial iris muscle of different species to adrenalin administration may not be identical. It is perhaps noteworthy that while the predomi-

nant effect of adrenalin on the radial muscle of the ox iris was relaxation, there occasionally was a double response, viz., a slight initial contraction followed by a marked relaxation, as may be seen on close examination of figure 4, part 1.

The finding that adrenalin administration may cause a double response in isolated radial and sphincter muscle fibres of the iris suggests that the action of this sympatho-mimetic substance is perhaps not a fixed and invariable one. In search for a possible explanation of this double response one is reminded of the influence of the autonomic nerves on the stomach where the response to nerve stimulation is conditioned to some degree by the state of the tissue at the moment so that sympatho-mimetic substances which give inhibitory effects on contracted muscle may also elicit excitatory effects on the relaxed muscle (McSwiney, 1931). The evidence that a similar relationship may obtain in the iris muscles, however, is by no means conclusive.

The sensitivity of the isolated radial muscle of the iris to sympatho-mimetic substance is apparently much lower than is that of the sphincter muscle. This is of interest in connection with the problem of pupillary regulation, for in those eyes in which the sphincter and dilator muscles are affected oppositely by adrenalin, viz., relaxation in sphincter and contraction in radial muscle, this difference in sensitivity would indicate that alteration in pupillary size induced by sympatho-mimetic substance is accomplished for the most part by changes in the tonus of the sphincter fibres. So far as the sympathetic nervous control of pupillary dilatation is concerned, the results of our *in vitro* experiments suggest that pupillary dilatation is accomplished by a predominant relaxation of the sphincter muscle, and that while such dilatation may be facilitated by a concomitant active contraction of the radial muscle, this contraction is not essential to the dilatation. In fact pupillary dilatation may very well occur even though the radial tonus remains constant or is actually decreased but to a lesser degree than is that of the sphincter; in this case a passive stretching of the radial fibres might be of significance.

Acetylcholine and radial fibres. Acetylcholine for most part was found to have no effect on the radial muscle, but to this general finding there were some few exceptions such as that shown in part 4 of figure 4, where administration of this parasympatho-mimetic substance caused a contraction.

If the effects of acetylcholine on isolated preparations may be taken as any index of the parasympathetic influence *in vivo*, it would appear from the results of our experiments on both radial and sphincter muscles of the iris that pupillary constriction arising from increased activity of parasympathetic endings (*in vivo*) is accomplished by variable degrees of contraction of the sphincter, without a necessarily concomitant active relaxation of the radial fibres. In fact there is evidence (fig. 4) which

indicates that under some conditions the parasympathetic supply, which is motor to the sphincter muscle, may also cause a distinct active contraction but of lesser degree in the radial muscle fibres.

SUMMARY

The inhibitory action of adrenalin on the isolated iris sphincter was confirmed. There were, however, some exceptions to this predominant finding, e.g., sympatho-mimetic substances as adrenalin may occasionally cause a contraction of the sphincter, or a double response made up of an initial relaxation followed by contraction.

While acetylcholine did elicit contraction in the isolated iris sphincter muscle, the sensitivity of this tissue to acetylcholine judging from the magnitude of response was, with few exceptions, found to be low. This low sensitivity contrasted with the relatively high sensitivity of the sphincter to inhibitory sympatho-mimetic substances is suggestive that the major control of the sphincter muscle *in vivo* is vested in its sympathetic innervation.

Iris muscle was found to be possessed of an inherent spontaneous rhythmicity which was most prominent during those periods in which the muscle was in a partially relaxed state. This inherent rhythmicity was stressed as a possible contributor to hippus which heretofore has been explained as of central origin.

The predominant action of adrenalin on isolated iris radial muscle in the dog and rabbit was found to be one of contraction, whereas in the beef eye it most frequently was one of relaxation. The exceptions to the generally accepted action of sympatho-mimetic substances on the sphincter and radial iris fibres were found to be prominent enough to warrant questioning whether there is not some fundamental process—perhaps in the neuromyal junction which determines just which of the reactions, contraction or relaxation, is to predominate.

The sensitivity of the dilator muscle appears to be much lower than that of the sphincter to both sympatho-mimetic and parasympatho-mimetic substances.

Evidence was cited in support of the belief that in so far as sympatho-mimetic substances are concerned the pupillary size is regulated largely through the sphincter component.

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STUDIES ON THE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE TOOTH ENAMEL OF EXPERIMENTAL ANIMALS[1]

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Aside from the well known destructive processes which affect the enamel of the teeth, it is generally believed that this tissue is unique in its lack of post-eruptive changes. Thus Chase (1931), in an extensive review of the controversy concerning the metabolism of the enamel, concludes that "the enamel is a lifeless, inert, mostly inorganic, substance." This conclusion was based on a critical consideration of the evidence available from numerous histological, chemical and physical studies of the enamel.

During the last decade, the use of radioactive isotopes, with which minute mineral metabolism can be measured, has opened a new approach for studying the metabolic changes of the dental hard tissues in the living organism. Chievitz and Hevesy (1935), using rats as experimental animals, applied this method for the first time to the study of phosphorus exchange in the teeth. Similar studies have been reported by Manly and Bale (1939). However, in neither case was the enamel studied separately. In further work with the isotope Hevesy, Holst and Krogh (1937) attempted to study the radiophosphorus metabolism of the enamel after separating it from the dentin by ignition, but their results were inconclusive. Hevesy and Armstrong (1940) reported that the exchange of radioactive phosphorus per gram of enamel was 6.7 to 10 per cent that of the dentin. Based on in vitro tests, they concluded that the radiophosphorus of the enamel was not acquired from the saliva. Simultaneously, Volker and Sognnaes (1940), on the basis of an in vivo study, reported that the enamel of a cat fed radiophosphorus attained a higher concentration of radiophosphorus in the surface layer than in the remaining portion of the enamel and suggested that this higher surface activity was acquired from the saliva. During the past year our studies have been extended to a greater number of animals, and an attempt has been made to determine the radiophosphorus metabolism in various parts of the enamel.

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MATERIAL AND PROCEDURES. The metabolism of P^{-32} in the enamel has been studied in 8 cats, 5 dogs, and 1 monkey. Radioactive phosphorus was obtained from the Department of Physics of this University, through the courtesy of Dr. S. N. Van Voorhis. The isotope P^{-32} has a half life of 14.5 days, and is prepared by bombarding red phosphorus with deutrons in the cyclotron. The nuclear reaction is as follows:

$$P_{15}^{31} + H^2 \rightarrow P_{15}^{32} + H^1$$

The cats used in the first series of experiments were fully grown, weighing from 5.2 to 7.7 pounds. They received the radioactive isotope by stomach tube as a solution of Na_2HPO_4 containing approximately 10 mgm. of the solute with a P^{-32} radioactivity varying from 400,000 to 3,000,000 counts per minute on the Geiger-Müller scale-of-four counter. After 1 to 9 days the animals were sacrificed and the jaws and teeth cleaned, dried at

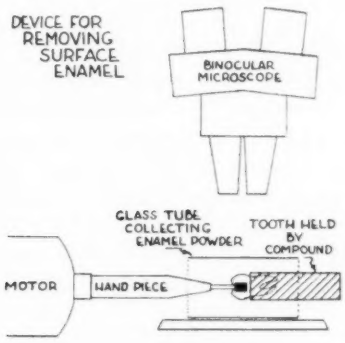

Fig. 1

110°C. in an oven, washed and separated. A device was constructed to permit us to grind off the surface layer of the enamel. This was accomplished under binocular microscope with the aid of a diamond stone and the enamel grindings were collected in a glass tube (see fig. 1). The remaining enamel was separated from the dentin by powdering of the teeth followed by the centrifugation-flotation separation of Manly and Hodge (1939), using a liquid of 2.80 density which assured us of enamel samples of high purity. The average weight of the enamel samples, obtained from the cats by these techniques, was 20 and 40 mgm. respectively for the surface layer and the remaining enamel.

In the second series of experiments, 5 adult dogs, weighing from 24 to 42 pounds were prepared for acute experiment under dial-urethane anesthesia. Solutions of radiophosphorus, containing approximately 10 mgm. of Na_2HPO_4 with a radiophosphorus activity of from 2.5 to 4 million

counts per minute, were introduced directly into an isolated part of the small intestine. Four hours later the animals were sacrificed and the various enamel samples prepared for an examination of their radioactive phosphorus content.

In dog 1 an attempt was made to study the distribution of radiophosphorus in the various density fractions of the enamel and dentin of the entire dentition. The separation of the enamel and dentin into the various density fractions was accomplished by a slight modification of the centrifugation-flotation procedure. With dogs 2 and 3 the procedure was essentially the same as with the cats of the first series, the outer layer of the enamel being removed by grinding with diamond stone, and the remaining enamel being separated from the dentin by the usual method.

In the experiments on the last two animals in this series, dogs 4 and 5, special emphasis was placed on the effect of the contact of the enamel surface with salivary secretions containing the radioactive isotope. To accomplish this, the teeth of dog 4 were completely covered with specially fitted metal trays, fixed to the dried teeth by means of 1 layer of impression compound and 1 layer of plaster. At the termination of the experimental period, the teeth were divided into two equal groups. The whole enamel from the first group of teeth was separated from the dentin by flotation and then further divided with the same technique into two fractions, one having a density greater than, and the other less than 2.90. The enamel from the second group of teeth was divided into three parts, the first two of which were prepared by successive surface grindings. Special care was taken in obtaining these samples to prevent inclusion of tooth substance from the cervical region where the enamel is thin and the possibility of dentin contamination is greatly increased. A device similar to that applied to the dentin of dog 4 was used to cover the teeth on the left side of the mandible and maxilla of dog 5. The teeth on the right side remained uncovered and were in continuous contact with saliva, the secretion of which was increased by electric stimulation of the chorda tympani nerve on the same side. Samples of surface enamel, remaining enamel, and crown and root dentin from the covered and uncovered side were compared for P^{-32} content. Periodic determinations of the radiophosphorus content of the saliva were made and at the completion of the experiment the radioactive phosphorus content of the stimulated and unstimulated submaxillary gland was determined. Since the dogs used in the second series of experiments were fairly large, it was possible to obtain appreciable samples of the surface enamel, the average weight of the surface enamel samples being approximately 75 mgm.

To date, one young adult monkey has been successfully studied. This animal was given a solution containing 10 mgm. of Na_2HPO_4 and 750,000 counts of radioactive phosphorus by stomach tube. A week later the

animal was sacrificed and the enamel of the erupted teeth and 4 unerupted third molars prepared for examination. The roots of the unerupted third molars were incompletely formed. Although the crowns appeared to be fully outlined, the enamel seemed to be in the immature stage, probably positive birefringent enamel, in contrast to the negative birefringent enamel of the mature erupted teeth. The enamel of the erupted and unerupted teeth was separated from the dentin by centrifugation-flotation technique and analyzed for radiophosphorus activity.

Two in vitro experiments were attempted. In the first of these, dog saliva was collected with the same technique as used in the experiment with dog 5. Approximately 700 counts per minute of radiophosphorus were added to 10 cc. of the saliva sample. The artificial mixture was comparable to the composition of the saliva observed in vivo in dog 5, with respect to amount and radioactivity. The mixture was allowed to come in contact with the enamel surface of six freshly-extracted dog teeth for a comparable time (4 hrs.) at body temperature. Two such experiments were run parallel. At the termination of the experimental period, the teeth were thoroughly washed and cleaned and the surface enamel removed by grinding, and analyzed for P^{-32} content.

Human teeth and saliva were utilized in the second in vitro experiment. The crowns of 10 freshly extracted, non-carious teeth were immersed in 20 cc. of fresh saliva to which 15,000 counts per minute of radiophosphorus and a few drops of toluene had been added. Twelve hours later, the teeth were removed from the saliva, washed for 4 hours in continuously running water, and the surface enamel prepared for counting, as in the previous experiment.

The tooth samples from all the experiments were dissolved in 2 cc. of 6 N HCl and their radioactive isotope content determined by the Geiger-Müller counter. When samples with low radioactivity were counted, a background count of distilled water was determined between each, or every second sample.

Results. The radioactive phosphorus distribution in the entire dentition, and its component parts, has been studied in detail and will be reported elsewhere (Volker and Sognnaes, 1941). To indicate the relationship of the P^{-32} metabolism in the enamel, as compared to that of the whole teeth, it will suffice to say that 1, the average weight of the entire dentition of the cats used in the first series was 2.2 grams, and approximately 0.02–0.05 per cent of the total dose administered could be found in the whole dentition. 2. The average weight of the entire dentition of the dogs used in the 2nd series was 30 grams and approximately 0.07 to 0.12 per cent of the total dose administered could be found in the whole dentition. 3. The teeth of the monkey weighed 11.7 grams and contained 0.06 per cent of the total experimental dose. It should be noted that the enamel probably

represents 10 to 20 per cent by weight of the full calcified teeth, and that the total weight of the dogs' teeth was approximately 0.2 per cent of the body weight as compared with 0.06 per cent in the cats.

In the cats, where only small enamel samples could be obtained, several samples counted less than twice the background and are of little significance in themselves. However, the ratio between the surface enamel and the remaining enamel, with respect to their radioactive phosphorus content, may be of considerable interest. The surface enamel in all the cats examined showed a higher P^{-32} concentration than the remaining enamel being from 1 to 9 times as high. The average per cent of the total dose per gram of surface enamel was 12.3×10^{-3}, as compared with 3.9×10^{-3}

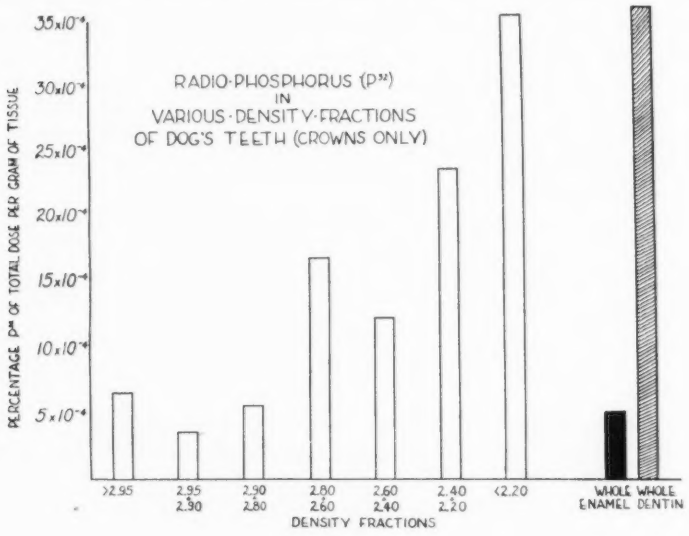

Fig. 2

for the remaining enamel, or a ratio of 3.1 to 1. The results are shown in table 1.

The distribution of the isotope in the various density fractions of the dental hard tissues of dog 1 is shown in figure 2. The concentration in the dentin is inversely proportional to the density, while the opposite seems to hold for the enamel. In the fraction with density 2.60 and 2.80, possibly the dento-enamel junction, there appears to be a concentration of the P^{-32}.

Findings with dogs 2 and 3 substantiated our observation in the cats. The surface enamel of dog 3 contained twice as much, and in dog 2 five times as much radioactive phosphorus as the remaining enamel.

The radiophosphorus content of the enamel of dog 4, whose teeth were protected from salivary contact, may be seen in table 2. The distribution of the activity was the reverse of that found in the cat series and in dogs 2 and 3, being lowest in the surface enamel and increasing as the dento-enamel junction was approached. In the whole enamel sample, which was separated into density fractions of greater and less than 2.90, the greatest concentration of activity was found in the lightest fraction of the enamel.

Table 3 shows the relative distribution of radiophosphorus in the dental hard tissues of covered and uncovered teeth of dog 5. The radiophosphorus content of the saliva and the salivary glands is also shown. The P^{-32} content of the surface enamel of the teeth on the covered left side of the jaw is negligible, while the surface enamel from the uncovered right side is approximately 60 times higher in activity. A comparison of the

TABLE 1

Radiophosphorus exchange in the enamel of cats' teeth

EXPERIMENTAL PERIOD	PERCENTAGE P^{-32} OF TOTAL DOSE PER GRAM OF		$\frac{\text{SURFACE E}}{\text{REMAIN. E}}$ RATIO
	Surface enamel	Remaining enamel	
days	($\times 10^{-3}$)	($\times 10^{-3}$)	
3	10.8	1.2	9.0:1
6	9.6	1.4	6.8:1
4	11.4	2.2	5.2:1
2	2.6	2.2	1.2:1
9	6.0	2.6	2.2:1
1	9.2	2.8	3.3:1
6	16.1	6.3	2.5:1
2	33.3	12.5	2.6:1
Average.....	12.3	3.9	3.1:1

remaining enamel samples from the covered and uncovered teeth separated by the flotation, reveals no marked difference, being only slightly higher on the right side of the jaw. The radiophosphorus concentration of the density fractions between 2.60 and 2.80, probably the dento-enamel junction, is unexplainable higher in the teeth of the uncovered left side. The difference between the coronal dentin and root dentin on the two sides of the jaw is negligible, giving a good check on the method. Of the submaxillary glands, the right gland, which was stimulated, was heavier than the left, unstimulated gland. In addition, the right gland showed a three times higher radioactivity per gram of tissue than the left gland. The rate of salivary secretion of the isotope, which is included in table 2, shows almost a two-fold increase between the second, third and fourth hour.

The results of the monkey experiment are seen in table 4. The radiophosphorus content of the enamel and dentin of the unerupted teeth is

TABLE 2

Radiophosphorus exchange in the enamel in absence of saliva

(Dog 4)

	PER CENT TOTAL DOSE PER GRAM OF TISSUE
Surface enamel	
1st layer	1.76×10^{-4}
2nd layer	3.73×10^{-4}
Remaining enamel	6.12×10^{-4}
Whole enamel	
Density > 2.90	1.89×10^{-4}
Density < 2.90	7.62×10^{-4}

TABLE 3

Radiophosphorus exchange in the teeth as influenced by saliva

(Dog 5)

TYPE OF SAMPLE	PERCENTAGE P^{-32} OF TOTAL DOSE	
	Left jaws (covered)	Tights jaws (stimulated saliva)
	(Per gram $\times 10^{-4}$)	
Surface enamel	(0.37)	23.0
Remaining enamel (density > 2.80)	1.65	2.95
D. E. junction (?) (dens. 2.80–2.60)	6.67	2.19
Crown dentin (density < 2.60)	41.5	42.8
Root dentin	54.9	55.2
Submaxillary gland	15.65	46.50
	(Per cc. $\times 10^{-4}$)	
Mixed saliva,		
After 1 hour	(Teeth covered)	10.71
After 2 hours	(Teeth covered)	12.80
After 3 hours	(Teeth covered)	24.90
After 4 hours	(Teeth covered)	44.90

TABLE 4

Radiophosphorus in erupted and unerupted monkey teeth

CONDITION OF TEETH	PER CENT P^{-32} OF TOTAL DOSE PER GRAM		$\frac{\text{ENAMEL}}{\text{DENTIN}}$ RATIO
	Enamel	Dentin	
Erupted	0.7×10^{-3}	5.0×10^{-3}	1:7.14
Unerupted*	23.0×10^{-3}	27.0×10^{-3}	1:1.17

* Developing third molars of same animal.

greatly in excess of the same tissues from the erupted teeth. The activity of the unerupted enamel is almost equal to that of the unerupted dentin, while in the fully erupted teeth the enamel has only one-seventh the activity of the dentin.

That the comparatively high radiophosphorus activity observed in the surface enamel was derived from the saliva, is further substantiated by the in vitro experiments. The dog surface enamel in the first in vitro experiment showed an activity of 99 counts per minute of radioactive phosphorus per gram of surface enamel, as compared with 72 counts per minute observed in the comparable in vivo experiment with dog 5. In the second, or human in vitro experiment, the surface enamel had a higher relative radiophosphorus activity of 365 counts per minute per gram of tissue.

Discussion. It is probable that in these experiments the radiophosphorus found in the enamel and dentin of the adult teeth represents an exchange rather than a deposition of phosphorus in the dental hard tissues. Unfortunately these are short term experiments and this hypothesis could not be tested experimentally.

The finding of a consistently higher concentration of radiophosphorus in the surface enamel, than in the remaining enamel, is in keeping with our earlier report (1940). Two possible explanations may be offered for the wide discrepancies between the surface enamel to remaining enamel ratios of the individual animals. In the first place, the rate of salivary secretion of the isotope is probably an important factor in the P^{-32} metabolism of the surface enamel, and might vary considerably in the different animals. Secondly, the microscopic grinding of the tooth surface is not a quantitative procedure, and it is possible that in some cases a fraction of the remaining layer of enamel, with a relatively lower P^{-32} content, was removed by grinding and included in the surface enamel sample. This would result in a decreased surface enamel to remaining enamel ratio.

The failure to find appreciable quantities of radioactive phosphorus in the surface enamel of the covered teeth of dogs 4 and 5 is strong evidence that the comparatively high concentration of P^{-32} in the surface enamel is of salivary origin. Additional confirmation of this belief may be found in the enamel radiophosphorus absorption studies of Manly and Levy (1939), and Armstrong (1940), who have indicated that powdered enamel may absorb inorganic phosphate from solution.

The experiments with dog 1, where the teeth were exposed to the saliva, indicate that the high density fraction of enamel under these conditions, has the greatest concentration of radiophosphorus. The amounts of P^{-32} in the high density fraction are of the same magitude as that found in the surface enamel of dog 2 and dog 3. This suggests that the surface enamel fraction and the high density fraction enamel are almost identical. Further support for this belief can be found in the experiments with dog 4,

where the surface enamel from the covered teeth of one side of the jaw shows a concentration of radiophosphorus which is almost identical with the enamel of the highest density fraction from the teeth of the remaining covered side.

The comparatively large amount of P^{-32} in the unerupted enamel of the monkey dentition, indicates that although microscopically the calcification of the enamel is well advanced, the radiophosphorus metabolism still continues at an accelerated rate. It is probable that two processes, one of deposition of phosphorus, the other an exchange of phosphorus, are proceeding simultaneously. This portion of the study has important bearing on the subject of tooth calcification, and is being extended.

SUMMARY

Following systemic administration of radioactive phosphorus, the distribution of the isotope in the enamel of 8 cats, 5 dogs and 1 monkey has been studied. The radiophosphorus metabolism in enamel of fully erupted teeth is of a considerably smaller magnitude than that found in the dentin. The greatest concentration of P^{-32} in the enamel was observed in the surface layer. The results indicate that the enamel is subject to a mineral metabolism, partly from within, via the pulp and dentin, and partly from without by contact with the oral secretions.

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SECRETINASE IN BLOOD SERUM

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When secretin is injected intravenously under conditions permitting observation of the pancreatic response, it is noted that there is a latent period of about two minutes, followed by a flow of pancreatic juice, which is at first brisk and then tapers off in a space of time varying with the amount of material given (1). The flow becomes increasingly slow and finally ceases. The gradual diminution of pancreatic response is clearly an index of disappearance of the hormone from the circulation. This may be due to any of the following factors: *a*, breakdown of the material by a secretin-destroying enzyme in the blood and tissues; *b*, excretion through the kidneys; *c*, storage or ingestion by certain cells. It is obvious that storage in the body is a question which can not be investigated. We have previously noted that urine concentrates so prepared that they should contain any secretin present are lacking in pancreas-stimulating activity, so that excretion of the material in the urine is unlikely. The question of secretin destruction by the blood and tissues has never been investigated, except for the established fact (2, 3) that it does not survive treatment with the secretions of the gastro-intestinal tract and therefore is ineffective when given orally.

In the present communication we wish to report our investigation of the possibility that destruction takes place in the blood and the mechanism whereby such destruction might take place.

EXPERIMENTAL. Anesthetized dogs were prepared in the usual manner. The secretin solution was made up to the strength necessary for 0.5 cc. to stimulate the pancreas to secrete 20 to 60 drops. The following studies were made.

1. *Effect of various blood constituents and of time of incubation.* Blood was withdrawn from the animals and incubated with the secretin solution in the volume proportion of 9 to 1, *i.e.*, 4.5 cc. of citrated whole blood, citrated plasma, serum, or a 50 per cent saline suspension of washed red cells and 0.5 cc. of secretin solution. Thus the injection of 5 cc. of the mixture contained the equivalent of the same amount of secretin as was present in the control injections of 0.5 cc. which were given periodically

during all experiments. The latter procedure was designed to control any spontaneous variations in the responsiveness of the animals. The incubation temperature was 37°C. In some experiments the plasma or serum was heated to 60°C. for half an hour, cooled, and then incubated with the secretin.

2. *Effect of temperature of incubation.* A large quantity of dog blood was withdrawn, permitted to clot, centrifuged, and the serum separated and incubated with a secretin solution in the same ratio used in the previous experiment at temperatures varying from 0°C. to 60°C.

3. *Effect of enzyme concentration.* The secretin solution was incubated for a definite time at 37°C. with varying volumes of dog serum in ratios of 1:1 up to 1:40, and injections of these mixtures were made in the amount equivalent to the secretin content of the control injections.

4. *Effect of hydrogen ion concentration.* By means of a Coleman pH electrometer samples of serum were brought to varying pH's ranging from 4 to 10. Samples of this adjusted serum were incubated with secretin solution for a definite time at 37°C., and injected.

Results. Incubation of secretin with whole blood, serum or plasma resulted in a decrease in activity on the pancreas which varied with the time of incubation. There was little or no secretin destruction after incubation with a suspension of washed corpuscles or with previously heated plasma and serum. The findings in detail are given in table 1. The effect of varying the temperature of incubation was evidenced by a relatively slight secretin-destroying activity at 0°C., which increased rapidly as the temperature was increased and became maximal at 37°C., then fell off and completely disappeared at 60°C. The data are listed in table 2. Varying the amount of serum with which the secretin was incubated showed that the extent of secretin inactivation depended upon the quantity of serum present. In table 3 the results of these experiments are detailed. The secretin-destroying activity of serum was found to be profoundly affected by the hydrogen ion concentration, and effective only in the range of pH 5 to 8 (table 2). At a pH outside of this range there was no destruction of the secretin by serum: however, it was possible to maintain serum at a more acid or more alkaline reaction, then to readjust the pH to the physiologic normal, incubate with secretin, and obtain inactivation.

Discussion. An examination of the data obtained reveals that dog's blood contains a substance which inactivates secretin. This principle was detected in the whole blood, plasma, and serum. There was little inactivation by washed corpuscles (dogs 5 and 8) and such slight effect as was obtained was probably due to insufficient washing. The inactivating potency was entirely destroyed by heating to 60°C. for 30 minutes; at this temperature there were no observable physical changes in the serum. When the temperature of incubation was varied, it was noted

that body temperature was optimal, and the extent of secretin inactivation depended on the amount of secretin-destroying substance present. The potency was operative in a rather narrow pH range. The agent was inactivated, but not destroyed, outside of this pH range. All of these findings point to the existence of an enzymic mechanism of secretin de-

TABLE 1

Showing the effect of dog's blood and its constituents and of incubation time on secretin

DOG NO.	PROCEDURE	RESULTS							DOG NO.	RESULTS						
1	Incubation time, min.	5	10	20	40				2	30	60	90	120	180		
	Control secretion, drops	28	28	31	31					43	48	45	43	43		
	Secretin + whole blood, drops	19	15	13	6					38	36	25	20	14		
3	Incubation time, min.	60	90	120	180	270	300		4	15	30	45	60	120	180	
	Control secretion, drops	43	51	51	62	63	62			20	21	19	19	20	19	
	Secretin + whole blood, drops	35	28	13	13	3	0			21	14	14	14	13	8	
6	Incubation time, min.	15	30	45	90	150	210	360	7	15	30	60	180			
	Control secretion, drops	20	17	19	18	18	17	18		28	30	20	31			
	Secretin + plasma, drops	18	13	11	9	8	4	0		26	19	12	14			
10	Incubation time, min.	15	45	60	90	120	180	1080	11	15	30	45	60	120	180	240
	Control secretion, drops	13	16	19	18	19	14	13		19	18	17	20	20	20	20
	Secretin + serum, drops	19	11	13	9	6	1	0		16	15	14	8	7	2	0
	Secretin + heated serum, drops						14	8						21		18
5	Incubation time, min.	15	45	120					8	15	45	60	90	180	240	
	Control secretion, drops	54	54	50						21	21	22	28	24	19	
	Secretin + plasma, drops	44	40	28						19	17	16	12	5	0	
	Secretin + cells, drops	51		43									28		15	
9	Incubation time, min.	15	30	45	60	120	210	300	25	30	60	120	180			
	Control secretion, drops	21	25	20	20	20	20	23		32	33	36	30			
	Secretin + plasma, drops	18	19	13	11	8	4	0		24	19	13	6			
	Secretin + heated plasma, drops				21			22		35	33	31	33			

struction. Until the enzyme has been demonstrated to be specific for a particular chemical group, it appears reasonable for convenience to call it secretinase.

A knowledge of the structure of secretin must of necessity precede any explanation of the mode of action of secretinase. The nature and mode of action are at present obscure, but it definitely does not act by proteolysis.

All the evidence at hand points to the non-existence of any proteolytic activity in untreated blood serum (4), and in this laboratory Doctor Beazell failed to detect any protein split-products after a 24-hour incubation of blood serum with a casein substrate. Agren and Hammarsten (5) incubated their secretin preparation with amino-polypeptidase, and while

TABLE 2

Showing effect of temperature and pH of incubation

DOG NO.	PROCEDURE	TEMPERATURE, DEGREES C.										
		0	10	12	15	23	25	30	37	45	50	60
12	Control secretion, drops	45				55						50
	Secretin + serum, drops	36	28			22		11	8			52
13	Control secretion, drops	39				39			49			
	Secretin + serum, drops	27			9				2	0	12	49
14	Control secretion, drops	6				4						5
	Secretin + serum, drops	4			3		0		0			4
15	Control secretion, drops	22				22				23		22
	Secretin + serum, drops	18		10		5			0	2	9	21

Effect of pH

DOG NO.	PROCEDURE	pH									
		4	5	6	6.5	7	7.5	8	9	9	10
21	Control secretion, drops	50				52					42
	Secretin + serum, drops	46	7	3		1		4		38	40
22	Control secretion, drops	30		30		27	19	19			
	Secretin + serum, drops	33	7	5	1	1	5	19			
23	Control secretion, drops	44					37	26	26		
	Secretin + serum, drops	37			25	22	24	15	25		
24	Control secretion, drops	26	35				27		26		
	Secretin + serum, drops	13*	34	16		7	3	7	24	14*	

* Incubated with secretin for 90 minutes at designated pH, after which pH was readjusted to 7.5, mixture reincubated for 1 hour and then injected.

they isolated free amino-acids in their hydrolysate from this treatment, they reported its secretin potency to be unaltered. On the basis of the general properties of the crystalline secretin described by us (6) we have concluded that it is unlikely that the molecular complexity of secretin is of great magnitude.

In order to substantiate this evidence we have incubated secretin with pure crystalline pepsin and trypsin[1] and noted no difference in the degree of inactivation produced by unboiled and boiled enzymes (table 4). Such inactivation as took place in the case of trypsin is explicable on the basis

TABLE 3

Showing effect of serum concentration

DOG NO.	PROCEDURE	SERUM VOLUME RATIO											
		1:1	2:1	3:1	4:1	5:1	6:1	7:1	8:1	9:1	14:1	20:1	30:1	40:1
16	Control secretion, drops	10	11	10	10		10							
	Secretin + serum, drops	8	6	4	1		1							
17	Control secretion, drops	72			70				68					
	Secretin + serum, drops	60	55		47		23		10					
18	Control secretion, drops	27		26		28								
	Secretin + serum, drops	19	14	9	4	1								
19	Control secretion, drops						36			36	36		36	32
	Secretin + serum, drops						22			17	13		10	2
20	Control secretion, drops	26		28		28				28		28		
	Secretin + serum, drops	18	14	9	12	4		5		4	5	1		

TABLE 4

Action of crystalline trypsin and pepsin on secretin

Incubation time, 4 hours

DOG NO.	PROCEDURE	RESULTS
24	Control secretion, drops	27
	Secretin + 0.7 mgm. trypsin at pH 8, drops	15
26	Control secretion, drops	27
	Secretin + 0.24 mgm. trypsin at pH 8, drops	21
	Secretin + 0.25 mgm. boiled trypsin at pH 8, drops	20
27	Control secretion, drops	18
	Secretin + 0.5 mgm. pepsin at pH 3, drops	19
	Secretin + 0.5 mgm. boiled pepsin at pH 3, drops	15

of the alkalinity of the solution. The fact that secretin is unaffected by proteases of such tremendous potency (checked in this laboratory by direct assay) provides conclusive evidence of its resistance to proteolytic enzymes.

[1] Obtained from the Plaut Research Laboratory, Bloomfield, New Jersey.

Our findings also demonstrate that the ineffectiveness of oral administration of secretin is not the result of its destruction by pepsin and trypsin in the gastro-intestinal tract. More probably the digestive secretions contain a secretinase similar or identical to that demonstrated by us in the blood.

We have examined one other possibility which, though remote, merits consideration—namely, that secretin might combine with some serum protein and be rendered ineffective by such a combination, whether physical or chemical. Obviously, according to our findings, such combination could not occur when the serum had been previously heated to 60°, or when it had been acidified or alkalinized outside the pH range of 5 to 9. In order to rule out the existence of such a process, secretin was incubated with serum for 3 hours, and portions of this mixture were heated to 60°, acidified to a pH of 1, and alkalinized to a pH of 9, and then assayed for activity. The results of this experiment are listed in table 5. If the hypothecated

TABLE 5

Action of heat, acid, and alkali on secretin-serum mixture after 3-hour incubation

Dog 27

INJECTION	RESPONSE	CONTROL RESPONSE
	drops	*drops*
Secretin + serum	7	24
Same heated to 60° for 30 min.	6	24
Same acidified to pH 1	6	26
Same alkalinized to pH 10	2	19

combination had taken place, the treatment given should have liberated the secretin and the treated mixtures should have been as stimulating to the pancreas as were the control injections. The fact that treatment with acid yielded no results is deemed particularly significant, since acid extraction is the procedure used for liberation of secretin from the intestinal mucosa; and these findings definitely settle the existence of secretinase.

Secretinase activity presents a complexity in its measurement. For example, in noting the effect of time of incubation, the enzymic inactivation of secretin takes a course which is at first gradual, later rapid, and finally gradual again. This circumstance is more apparent than real. We have previously noted (1) that pancreatic response is related to secretin dosage according to an S-shaped curve. For this reason it is not possible to plot concentration-action curves or time-action curves for the enzyme. A unit of secretinase may be defined, for convenience, as that quantity which will, in two hours' time, reduce the potency of two threshold doses of secretin to one threshold dose. The experiments cited above have shown

this to be roughly the average amount present in 4.5 cc. of dog's blood plasma or serum.

We have on several occasions attempted to determine the cause of variation in the responses of individual dogs to secretin. In certain refractory animals the reason is obvious in the form of outspoken pancreatic pathology; however, refractoriness has frequently been seen in animals with apparently healthy glands. In the series studied during the present work, three dogs (1, 10, and 11) were refractory. Dog 1 was particularly so. The blood of these animals appeared to be more potent in destroying secretin. Hence it appears that refractoriness may be due in part to a greater concentration of hormone-destroying enzyme in certain cases.

SUMMARY AND CONCLUSIONS

The incubation of secretin with dog's whole blood, plasma or serum has been found to inactivate the secretin. The principle responsible for the inactivation is operative within a narrow range of hydrogen ion concentration, acts most rapidly at body temperature, and is heat-labile. The extent of action depends on the time of incubation and the amount of blood used. These findings demonstrate the presence in the circulation of an enzyme which inactivates secretin and which for convenience will be called secretinase until it has been shown to be an enzyme specific for a certain chemical group.

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SEASONAL AND POSTURAL CHANGES IN BLOOD VOLUME DETERMINED BY A CARBON MONOXIDE METHOD, EMPLOYING A DIFFERENTIAL ELECTRIC PHOTOMETER FOR THE ESTIMATION OF LOW PERCENTAGE SATURATIONS OF HEMOGLOBIN WITH CARBON MONOXIDE

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Modern methods for the determination of circulating blood volume in man fall into two classes: 1, methods which determine the total amount of circulating hemoglobin, from which value the total circulating blood volume may be calculated, and with the use of the hematocrit ratio the red cell and plasma volumes, and 2, methods which measure the circulating plasma volume, from which value the red cell and total blood volumes are calculated with the use of the hematocrit ratio. Of the methods belonging to the first class, the one most widely used is that in which the total circulating hemoglobin is estimated by measuring the increase in the amount of carbon monoxide hemoglobin following the inhalation of a known amount of carbon monoxide. This method as developed by Haldane and Smith (1) had the disadvantage that rather high percentages (20 to 25 per cent) of carbon monoxide hemoglobin were required for measurement by the methods then available; the necessarily large amounts of carbon monoxide inhaled made the blood volume values obtained by this method somewhat unreliable, for they introduced the questionable factors of 1, asphyxia, and 2, the uptake of significant amounts of the gas by muscle hemoglobin and the body tissues in general. Of the methods belonging to the second class, that of measuring the dilution of a known amount of dye injected intravenously is the most widely used. This method was introduced by Keith, Rowntree and Geraghty (2) and in recent years has been so refined by Gregersen and Gibson (3) and by Gibson and Evans (4) using the dye T1824 and by Sunderman and Austin (5) using the dye congo red that it has become the standard research and clinical method. The chief disadvantages of this method are 1, the measurements of the dye

[1] In partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Doctor Chambers designed and supervised the building of the electrical parts of the apparatus.

concentrations are complicated if either hemolysis or lipemia is present, and 2, repeated determinations at short intervals may result in undesirable discoloration of the subject. In addition, the question of the loss of some of the dye to the lymph (e.g., in the liver) is still unsettled.

In a series of papers reporting the results of a careful study of the relative values of the different methods for blood volume determinations, Whipple and his associates (6, 7, 8) came to the conclusion that for accurate estimation of circulating blood volume it is necessary to make simultaneous measurements by both the carbon monoxide and dye methods, for only the red cell volume can be measured accurately by the former, the plasma volume by the latter, method. For the total blood volume, the two values obtained independently but simultaneously should be added.

In recent years the carbon monoxide method has been revived due to the development of gasometric and photometric methods which permit accurate measurements of small percentages of carbon monoxide hemoglobin. These techniques make it possible to determine total circulating hemoglobin after inhalation of relatively small amounts (100 ml. or less) of carbon monoxide, which small amounts minimize any errors resulting from partial asphyxia or from the uptake of the gas by the tissues and muscle hemoglobin.

It is the purpose of this paper to present data on blood volumes obtained by a carbon monoxide method, the general procedure of which is essentially that of Chang and Harrop (9). The measurements of the percentage of carbon monoxide hemoglobin and of the hemoglobin concentration were made with the use of a differential electric photometer, the outstanding advantage of which is that accurate determinations of low percentages (5 to 12 per cent) of carbon monoxide hemoglobin may be made on very small amounts (0.04 to 0.4 ml.) of whole blood, thus making it possible to do repeated determinations of blood volume (once or twice daily) on the same subject. Some comparisons have been made by simultaneous estimates by both carbon monoxide and dye and the two methods give values which do not differ greatly (10).

METHODS. *Preparation and Measurement of Carbon Monoxide.* The carbon monoxide was made by the action of concentrated sulphuric acid with sodium formate. After passage through a 15 per cent solution of sodium hydroxide the gas was stored under positive pressure over water (containing alkali) in a 10 liter bottle. After each renewal of the store the gas was analysed for its oxygen content by means of a Haldane gas analyser, and correction of the carbon monoxide percentage was made on the assumption that the only contaminating gas was air.

The gas to be inhaled was collected under positive pressure over water in a 100 ml. burette (fig. 1) where it could be stored and measured. For its administration, the gas was forced into a Sanborn closed-circuit metabolism apparatus, following which 1 to 2 liters of oxygen were passed into the Sanborn by way of the two upper sidearms (*1* and *2*) of the burette, thus carrying along the carbon monoxide left in the connec-

tions. Additional oxygen was added as needed, in quantities of about 500 ml. For short experiments (30 min. or less) the ordinary nose clips and mouth pieces were used. For longer experiments a small mask (Heidbrink type, Ohio Chemical Company) with little additional dead space was preferred because of its greater comfort for the subject.

The residual carbon monoxide in the lung-Sanborn system was estimated on the assumption that this system had a volume of 4 liters. This value must vary with different individuals and with the degree of emptying at the end of an experiment. On the basis of an actual analysis, wherein a sample of residual gas contained 0.12 per cent of carbon monoxide and a sample of the blood contained 12.55 per cent carbon monoxide hemoglobin, the volume of carbon monoxide unabsorbed was only 5 ml. if a lung-Sanborn volume of 4 liters was assumed. We have considered it sufficient to calculate the residual carbon monoxide as 0.01 × per cent COHb × 4 liters. In the experiments so far performed, it has varied between 3 and 5 ml. An error of 1 ml. in the estimation of the unabsorbed carbon monoxide would cause an error of about 1 per cent in the blood volume determination.

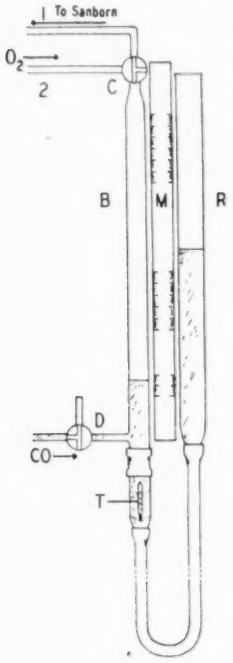

Fig. 1. Burette for measuring the volume of CO inhaled. *B:* measuring burette. *R:* water reservoir. *M:* meter stick. *T:* thermometer. *C* and *D:* 3 way cocks.

Colorimetric Estimation of Hemoglobin Concentration and of the Percentage Saturation of Blood with Carbon Monoxide. The presently accepted principles underlying the colorimetric determination of the percentage concentration of carbon monoxide hemoglobin were developed originally by Vierdordt (11) and by Hüfner (12). The theory involved is discussed in the articles, among others, of Butterfield (13), Ray, Blair and Thomas (14) and Hartmann (15).

The *differential electric photometer* was designed for the measurement of low concentrations (5 to 12 per cent) of carbon monoxide hemoglobin, but we have reason to believe (unpublished data on dye concentrations in serum) that it is readily adaptable to colorimetric determinations of other substances. The system (fig. 2) consists of a high pressure mercury vapor lamp (General Electric AH4) so arranged that it throws two beams of light in opposite directions. To minimize the variations when the lamps are changed and to confine the beams within the boundaries of the sensitive surfaces of the photocells, the light utilized is limited to that from the center of the vertical cylindrical arc. This is accomplished by a horizontal slit in the path of each beam about 2.5 cm. from the mercury arc itself. Each of the two beams, after passing through monochromatic filters, falls

on one of a pair of caesium oxide photocells, one placed at either end of a 30 inch optical bench. The two photocells oppose each other in a differential circuit, their net current being taken through one stage of amplification to an electric eye which is used as a null point indicator. The electric

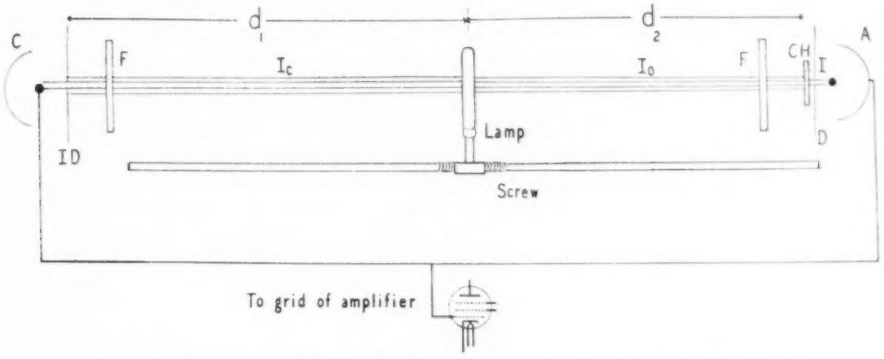

Fig. 2. Schematic diagram of the apparatus. *C* and *A:* "control" and "active" photocells, respectively. *ID:* adjustable iris diaphragm. *D:* a fixed diaphragm. *CH:* holder containing solution or gray glass. *F:* filters.

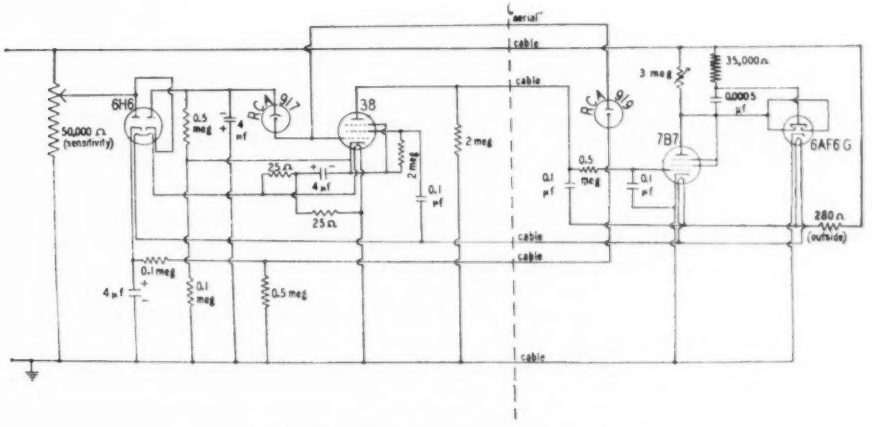

Fig. 3. Diagram of the electrical circuit

circuit is a modification of one presented by Shepard (16) and is shown in figure 3.

The mechanical arrangement. It must be emphasized at the start that we believe the accuracy of our results depends largely upon the meticulous care with which the

machine was built.[3] The lamp, placed at the approximate center of an optical bench, is attached to an adjustable carrier which moves along a screw having an accuracy of 0.0002 inch in 8.0 inches. With the use of the carrier and an extension of the optical bench, the working distance between photocells may be varied from 50 to 30 inches. So far, 40 and 30 inch distances have been utilized. The distance of the lamp from its midpoint position towards the "active" photocell (i.e., the photocell before which the test solutions are placed) is measured by means of a series of 3 cogged disks which permit readings to be made to 0.0001 inch. At either end of the optical bench, and connected to the case of the mercury lamp by bellows, is an aluminum box, blackened within, each of which contains a photocell (RCA 917 in one box, 919 in the other), two filter holders, and a part of the electrical equipment (see broken line, fig. 3, for distribution of the electrical parts between the two boxes). In addition, the box containing the "control" photocell (that on which the light intensity depends only on the position of the lamp) has an iris diaphragm so that the intensity of the light falling on the photocell may be adjusted; the box containing the "active" photocell has three holders for the solution cells and a holder for a standard gray glass. The holder for the standard glass is fixed behind, and therefore moves with, the center

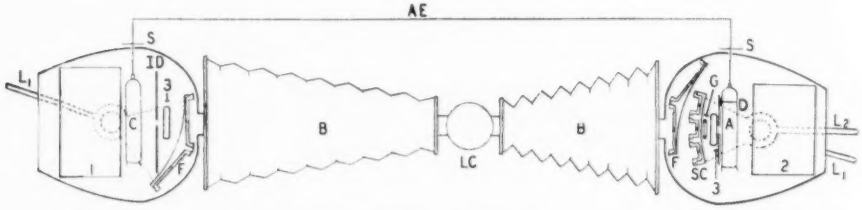

Fig. 4. Drawing of the apparatus looking down upon it. *1* and *2:* boxes containing the electrical equipment. *3:* $CuSO_4$ filters. L_1 and L_2: Lever controls for filters and solution cells, respectively. *C* and *A:* "control" and "active" photocells, respectively. *ID:* adjustable iris diaphragm. *D:* fixed diaphragm. *F:* filter holders. *G:* holder for gray glass. *SC:* holders for solution cells. *LC:* lamp case. *B:* bellows. *AE:* "aerial"—connection between photocells. *S:* light shields.

solution cell holder; the cell in this holder is filled only with the solution used to dilute the blood. The filter holders and the holders for the solution cells are arranged to rotate on pivots, being controlled from the outside of the boxes by levers whose positions are accurately fixed by stops (fig. 4).

The *filter combinations* used were as follows: 1. For the green line (546 mμ): 12 mm. thickness of 0.35 M $CuSO_4$ + Wratten 62 + Corning combination no. 5.1 + an additional 5 mm. didymium (Corning). 2. For the yellow lines (577 − 579 mμ): 12 mm. thickness of 0.35 M $CuSO_4$ + Zeiss A combination + 1 mm. Jena BG 7.

Theoretically the copper sulphate filters should be placed so that infra-red radiation is removed before reaching the solutions to be tested (there is some evidence that a change in temperature may disturb the proportion of carbon monoxide bound with hemoglobin). Practically in this position the $CuSO_4$ holders, unless absolutely optically flat and rigidly fixed in position, may cause difficulties through variable scattering of the light.

[3] The mechanical arrangement of the apparatus was designed and built by Carol Kelly.

It has been found necessary to use a voltage stabilizer (Sola) in both the lamp and amplifier circuits, but these may be unnecessary if the line voltage is steady. The case of each stabilizer, and of each aluminum box, must be thoroughly grounded.

We have recently replaced the RCA 917 and 919 photocells by the new RCA 929 photocells, which latter type appear to be superior to the former. They do not require any changes in the electric circuit. Due to their low sensitivity to infra-red radiation, the $CuSO_4$ filters may be unnecessary.

Use of the apparatus. With the lamp halfway between photocells, and no standard glass or test solution in the path of the light falling upon the "active" photocell, the intensity of the light falling upon the "control" photocell may be adjusted by the iris diaphragm until the null point is reached as indicated by the electric eye, i.e., the "net" current from the two photocells is zero. If now a standard glass or a test solution is placed before the "active" photocell, the current balance between the photocells is disturbed. To bring this "net" current back to zero, the *lamp* is moved nearer to the "active" photocell, until the electric eye again indicates the null point.

For calculation of the extinction coefficient (E) of the standard glass or of the test solution, use is made of the inverse square law of light intensity. If I and I_c represent the intensities of the light incident on the two photocells (see fig. 2), and I_o the intensity of the light incident on the standard glass:

$$I_o = k\,\frac{1}{d_2^2} \text{ and } I_c = k\,\frac{1}{d_1^2}$$

When balance is attained, $I = I_c$, so that $I = k\,\frac{1}{d_1^2}$. According to Lambert's law the extinction coefficient (E) of a solution of a standard thickness (1 cm.) is proportional to the logarithm of the ratio of the intensity (I_o) of the incident light to the intensity (I) of the transmitted light divided by the thickness (n) of the solution. That is

$$E = \frac{1}{n}\log_{10}\frac{I_0}{I} = \frac{1}{n}\log_{10}\frac{d_1^2}{d_2^2}$$

In practice, a graph is used to determine the value of $\log_{10} d_1^2 - \log_{10} d_2^2$. Then E can be calculated readily.

Theoretically, such calculations hold only for a point source of light. Practically, the light source in the present instrument is a band; in addition there are errors from reflections of the beams from the filters, cells, etc., and errors from the use of varying areas of the sensitive surfaces of the photocells.

In a simple system in the absence of any gray glass or solution the values of d_1 and d_2 would be those of the distances from the lamp to the photocell (fig. 2). In the complicated arrangement necessitated by the filters, diaphragms, etc., their values are found to be less. The value of the "effective length" of the system may be calcu-

lated by determining the positions of balance when glasses (preferably two or more) of known optical density, or stable solutions of known molecular extinction coefficients, are inserted between the lamp and the "active" photocell. This procedure is explained most readily by an example:

Let ΔD be the known optical density of a solution in a cell of thickness n and L the "effective length" of the system when the light is centered (i.e., in the absence of the absorbing solution or gray glass before the "active" photocell) and $d_1 = d_2 = L$. If X is the distance from the center point to the position of the lamp after balance is attained with the solution in place

$$\Delta D = nE = \log_{10} \frac{(d_1)^2}{(d_2)^2}$$

and $d_1 = L + X$

$d_2 = L - X$

from which L may be calculated. The value of L may differ at different positions of the lamp, but the values should not differ by more than 0.5 inch.

The actual determination of L is most readily attained by the use of standard neutral (gray) glasses or solutions of copper sulphate as recommended by Drabkin and Austin (17); in the latter case readings should be more preferably in the yellow line. Once L is known, the accuracy with which the inverse square law is followed may be tested by utilizing solutions of copper sulphate of different strengths. With solutions varying by 400 per cent read in a number of different cells the estimates of the molecular extinction coefficient showed a standard deviation of $\pm$ 0.4 per cent from the theoretical value. The discrepancies include those due to the glassware as well as those dependent on making the dilutions.

Procedure. The simplest, and most accurate, method of using the apparatus is to determine the optical density of the unknown solution as so much more or less than that of a neutral (gray) glass, the optical density of which is known and is about that of the solution to be tested. The neutral glass is placed behind the center solution cell (containing the solvent used to dilute the blood), and with the lamp close to its theoretical position (for the optical density of the glass), the iris diaphragm is adjusted so that the electric eye indicates the null point. Two readings are then made for the gray glass with one for the solution *between* them. After correcting for zero errors on the basis of any error in the setting of the gray glass, the optical density of the solution is calculated from the setting of the lamp. For estimates of the change in the optical densities of blood due to carbon monoxide, the above procedure is used alternately for the control and experimental bloods, two or more readings being obtained for each.

Deterioration of hemolysed blood is a source of error unless great care is taken in handling of the blood. When present it is usually accompanied by a definite and consistent change in the optical density of the solution which is not matched by a similar change in the other hemoglobin solution read at the same time. Where slight and approximately equal changes develop in both samples of blood during the period of measurement these changes are probably due to some effect (? thermal) on the system as a whole. They create only minor errors in the determination of the differences between the two bloods.

Preparation of the bloods. In order to make the determinations as accurate as possible, great care is taken in the preparation of the blood samples. By means of a "bulb" pipette of fine bore (calibrated to contain 0.4 ml.) a sample of heparinized whole blood is transferred from a clean, flamed, paraffined watch glass to saline in a 12 ml. centrifuge tube having a narrow calibrated neck. To minimize sedimentation

the blood is kept well agitated up to the taking of the sample. After centrifuging and removal of the supernatant saline, the cells are hemolyzed and diluted to the mark by 0.1 per cent Na_2CO_3. The resulting dilution is about 1 in 30. After thorough mixing about 1.5 ml. is withdrawn to create an air-pocket below the neck of the centrifuge tube. As it is essential that reduced hemoglobin be absent, the solutions are thoroughly oxygenated, after which they are spun to clear them of any debris.

The cells in which the solutions are read contain approximately 0.5 ml. of the solution, which has a thickness of about 1.0 mm.[4]

Estimation of hemoglobin concentration. For the determination of the hemoglobin concentration the extinction (E) of the blood solution in the green mercury line (546 mμ) is used. In this line the absorptions of oxyhemoglobin and of carbon monoxide hemoglobin are nearly identical (15, 18). The concentration "c" is calculated according to the Lambert-Beer law from the ratio of E to the specific extinction coefficient ϵ. For a solution containing 1 gram of hemoglobin per 100 ml. in a thickness of 1 cm., ϵ was found to have an apparent value of 8.34 for oxyhemoglobin (as determined by checking with a Van Slyke apparatus). Hartmann (15) used a value of 8.00 as given by Butterfield (13). Discrepancies are to be expected since the apparent value of this constant is affected by errors in the determination of the absolute thicknesses of the cells used as well as by impurities in the light. We have assumed that Hartmann is correct in estimating the optical density of pure carbon monoxide hemoglobin as 4 per cent less than that of pure oxyhemoglobin, giving, under our conditions, a ϵ value of 8.01 for carbon monoxide hemoglobin. Since the carbon monoxide hemoglobin saturation rarely exceeds 10 per cent any error in this assumption is unimportant in the determination of hemoglobin concentration.

The *consistency* of the estimations of hemoglobin concentrations is demonstrated in the results obtained on a number of duplicate samples (table 1 A). The standard deviation of the duplicates from their means is ±0.34 per cent for the 30 inch bench. In addition there are several sets of quadruplicate estimations (table 1 B). As may be seen some duplicates give good checks while a few are badly out. The most likely sources of trouble are errors in measuring the blood, particularly those created by air bubbles. It is to be emphasized that these deviations represent over-all errors, including pipette and dilution errors, sedimentation errors, reading errors, and personal errors as usually the samples were prepared by two different individuals. On a series of repeated readings of the same hemolysed sample (frequently read in different solution cells) made with a 30 inch bench, the standard deviation from the mean is ± 0.14 per cent. The actual reading error of the photometer is about ± 0.025 per cent. The main errors depend upon the handling of the blood samples and the cells that contain them.

Estimation of carbon monoxide hemoglobin. The present method involves the determination of the increase of carbon monoxide hemoglobin in the blood following the administration of carbon monoxide. The percentage of carbon monoxide hemoglobin in a sample of blood (taken after inhalation of the gas) is found by determining the *difference* in the value of its ratio $\Delta D_{546}/\Delta D_{578}$ (optical density for green line divided by that for the yellow) from the value of the corresponding ratio of the oxy-

[4]The thickness of the cell used as the standard was determined either 1, by mercury, or 2, by using a solution of copper sulphate with a known extinction coefficient and from its optical density calculating the thickness. Since the volume used to fill the cell is so small, the blood drawn can be reduced to 0.04 ml. and diluted to 1.2 ml. if desired. Under such conditions there is some loss of accuracy and considerably increased difficulties in handling.

hemoglobin control sample (taken before inhalation of the gas) read at the *same time*. The percentage of carbon monoxide hemoglobin which causes this difference in the ratios is read from a graph (fig. 5) which was made as follows:

A series of the theoretical values of the ratio $\epsilon_{546}/\epsilon_{578}$ for mixtures of different oxyhemoglobin and carbon monoxide hemoglobin percentages was calculated from the apparent ϵ values in the green and yellow lines for both pure oxyhemoglobin and carbon monoxide hemoglobin at 24°C. These are:

O_2Hb: $\epsilon_{546} = 8.342$, $\epsilon_{578} = 8.995$
COHb: $\epsilon_{546} = 8.008$, $\epsilon_{578} = 5.889$

TABLE 1

A			B		
HEMOGLOBIN CONCENTRATION		DEVIATION FROM THE MEAN OF THE PAIR	HEMOGLOBIN CONCENTRATION	MEAN	STANDARD DEVIATION FROM MEAN OF GROUP
Sample 1	Sample 2				
gms./100 ml.	*gms./100 ml.*	*per cent*	*gms./100 ml.*	*gms./100 ml.*	*per cent*
14.69	14.60	±0.31	1. 14.26		
15.28	15.28	±0.00	14.26	14.29	±0.37
16.06	16.05	±0.03	14.27		
15.46	15.32	±0.46	(14.37)		
15.14	15.13	±0.03			
16.19	16.20	±0.03	2. 12.81		
15.38	15.59	±0.68	12.86		
15.38	15.22	±0.52	12.84	12.84	±0.16
15.05	15.17	±0.40	12.83		
14.84	14.86	±0.07			
14.72	14.91	±0.64	3. 12.67		
13.97	13.95	±0.07	12.78		
13.82	13.89	±0.25	12.76	12.74	±0.39
15.09	15.08	±0.03	12.76		
14.55	14.51	±0.14			
14.49	14.40	±0.31			
14.43	14.45	±0.07			
14.28	14.41	±0.45			
14.46	14.46	±0.00			
Standard deviation of duplicates from their means: ±0.34 per cent.					

Thus in a mixture of 95 per cent O_2Hb + 5 per cent COHb the specific extinction ϵ is, in the green:

$$8.342 \times \frac{95}{100} + 8.008 \times \frac{5}{100} = 8.325$$

and in the yellow (similarly determined) is 8.839. The ratio $\epsilon_{546}/\epsilon_{578}$ of this mixture is thus 0.9418. Thus in the above example the ratio of 100 per cent oxyhemoglobin is 0.9274, and 0.0144 represents the change in ratio caused by 5 per cent of carbon monoxide hemoglobin. Figure 5 shows the changes in ratio produced by various saturations of hemoglobin with carbon monoxide.

Others (15, 19), utilizing a similar method have found that the ratio $\Delta D_{546}/\Delta D_{578}$ for the control blood varied with the blood of different individuals, and also with different blood samples from the same individual taken at different times. Such variations have been interpreted as due to the presence of various amounts of residual carbon monoxide hemoglobin before inhalation of the carbon monoxide. Variations occur (table 2) but are often dependent on other factors. Very small differences in the arrangement of the apparatus, in the lamp filters used, or in the temperature at which the bloods are read can cause considerable variations in the estimate of this ratio. A single blood, read in duplicate, may give different ratios. These changes appear most commonly to be due to a slight change in the temperature. Conse-

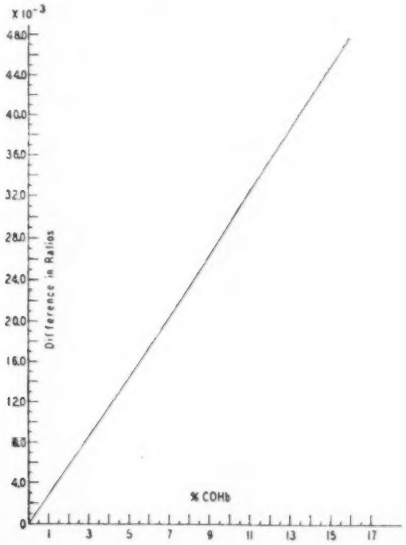

Fig. 5. Graph for estimating the percentage of carbon monoxide hemoglobin from the difference in ratios, at 24°C. Ordinate: $\Delta D_{546}/\Delta D_{578}$ of sample containing carbon monoxide hemoglobin $-\Delta D_{546}/\Delta D_{578}$ of control (oxyhemoglobin) sample. Abscissa: percentage of carbon monoxide hemoglobin. The ratios for pure O_2Hb and COHb, and consequently the graph, are slightly different for the RCA 929 photocells.

quently in the present form of the apparatus it is not possible to measure with exactness (nearer than 0.5 to 1.0 per cent) small initial concentrations of carbon monoxide hemoglobin in the control blood, nor to state that any observed small variations in the ratio are entirely dependent on contamination with carbon monoxide. The presence of such variations is, however, not serious in estimating the change produced by the inhalation of additional carbon monoxide, since the ratio for the carbon monoxide hemoglobin sample, *read at the same time* and under the same conditions, tends to change in the same direction. Consequently the difference between the ratios of the oxyhemoglobin and of the carbon monoxide hemoglobin samples is little affected by the absolute levels (table 3).

Examples of the effects of residual carbon monoxide are shown in table 2, which data represent the results obtained on the oxyhemoglobin (control) bloods of four students, two of whom were habitual smokers while the other two were non-smokers. Taking the average of the ratios of the non-smokers as a fair estimation of an oxyhemoglobin ratio, it was found that on the average the blood of one smoker contained 1.53 per cent, that of the other smoker 2.20 per cent, more carbon monoxide hemoglobin that did the bloods of the non-smokers.

The *consistency* of the determinations of carbon monoxide hemoglobin obtainable by this method is illustrated by the data presented in table 3. The standard devia-

TABLE 2*

$\Delta D_{546}/\Delta D_{578}$ *values obtained on control (oxyhemoglobin) samples of blood at different times from 4 subjects*

Subjects H and M: habitual smokers. Subjects K and L: non-smokers

SUBJECT H	SUBJECT M	SUBJECT K	SUBJECT L
0.90330	0.90617	0.90159	0.89772
0.90446	0.90536	0.89902	0.89759
0.90280	0.90699	0.89855	0.89778
0.89977	0.89900	0.89254	0.89987
0.90267	0.90271	0.89706	0.90039
0.90078	0.90232	0.89700	0.89405
0.90026	0.90269		0.89501
	0.90365		0.89460
			0.89766
			0.89917
			0.90035
			0.89790
			0.89838
Mean....0.90201	0.90361	0.89763	0.89773

* The low ratios here shown as compared with those given in the text were dependent on the optical densities assigned to the gray glasses employed. These densities were later adjusted according to values given by the Bureau of Standards. Though the actual values differ from those in use at present, any error introduced is small. The constants then utilized for hemoglobin concentration depended on a comparison with gasometric determinations, and those for carbon monoxide hemoglobin saturation on determination of the actual ratios obtained with oxyhemoglobin and carbon monoxide hemoglobin at that time.

tion of duplicates from their means is ± 0.82 per cent of the total saturation. The data of tables 1, 2 and 3 were obtained with a 30 inch bench. A longer bench (40 in.) increased the accuracy for estimation of hemoglobin concentration but decreased the accuracy of the estimations for the ratios $\Delta D_{546}/\Delta D_{578}$. This we attribute to the greater intensity of light available to the photocells with the shorter bench, giving a greater accuracy of reading in the yellow line which overbalances the increase in other errors.

Since the curve of figure 5 is nearly a straight line differences may be used to estimate the increase in carbon monoxide hemoglobin saturation without regard to the initial level of residual carbon monoxide provided that this does not exceed 3 to 4

per cent. If large initial concentrations are present the curve should be read at the appropriate level. In a case where the control sample appeared to contain 1.93 per cent carbon monoxide hemoglobin, the increase in the saturation of the blood following the inhalation of carbon monoxide was 10.10 per cent as determined by the simpler method, and 10.02 per cent when the curve was read at the proper levels. The difference is insignificant.

TABLE 3*

Data obtained on duplicate blood samples showing 1, the values of the ratio $\Delta D_{546}/\Delta D_{578}$ for the control (O_2Hb) samples and the samples ($COHb$) following the administration of CO; 2, the difference in the ratio for the COHb and its O_2Hb control, and 3, the percentage COHb samples corresponding to the difference

BLOOD NO.	$\Delta D_{546}/\Delta D_{578}$		DIFFERENCE	COHb
	O_2Hb	COHb		
				per cent
1 A	0.90330	0.92658	0.02328	8.33
1 B	0.90446	0.92775	0.02329	8.33
2 A	0.90159	0.92117	0.01958	7.08
2 B	0.89902	0.91840	0.01938	7.03
3 A	0.89772	0.92768	0.02996	10.70
3 B	0.89778	0.92768	0.02990	10.67
4 A	0.90617	0.92655	0.02038	7.37
4 B	0.90536	0.92598	0.02062	7.47
5 A	0.89987	0.92564	0.02577	9.23
5 B	0.90039	0.92619	0.02580	9.25
6 A	0.89254	0.92020	0.02766	9.88
6 B	0.89706	0.92412	0.02706	9.68
7 A	0.90078	0.92304	0.02226	8.01
7 B	0.90026	0.92225	0.02199	7.92
8 A	0.89790	0.92183	0.02393	8.57
8 B	0.89838	0.92213	0.02375	8.52
9 A	0.89565	0.92126	0.02561	9.17
9 B	0.89704	0.92244	0.02540	9.12

* See footnote table 2.

Standardization. Careful standardization of the apparatus under the conditions in which it is to be used appears to insure reliable results in spite of the variable values of the constants, and consequently of the ratios, which may be obtained under different conditions. Hartmann (15) obtained a ratio for pure oxyhemoglobin of 0.9458 and one for carbon monoxide hemoglobin of 1.401, while Steinmann (19) using essentially the same apparatus obtained values of 0.914 and 1.355 respectively. The differ-

ences in these ratios ($\Delta D_{546}/\Delta D_{578}$ COHb − $\Delta D_{546}/\Delta D_{578}$ O_2Hb) are, however, of the same order, 0.4552 and 0.441 respectively. With various slight modifications of the present apparatus values have been obtained for the ratios which have differed considerably, the pure oxyhemoglobin ratio ranging from 0.8920 to 0.9274, the pure carbon monoxide hemoglobin ratio from 1.3067 to 1.3598. In spite of the large variations in the absolute levels of the ratios, the values of the differences have varied by no more than 3 per cent (0.4147 to 0.4267).

The differences in ratios given by both Hartmann (15) and Steinmann (19) are greater than those calculable from the data of Heilmeyer and Sundermann (20) or Drabkin and Austin (18). With the RCA 929 photocells in the apparatus instead of the RCA 917 and 919 combination, the ratio at 24.3°C for oxyhemoglobin[5] is 0.90097, and for carbon monoxide hemoglobin 1.2978, with a difference of 0.3968, a value identical with the average value of 0.397 found by Heilmeyer and Sundermann. The RCA 929 photocells are not sensitive to infra-red; in our earlier arrangements, though the RCA 917 and 919 cells were sensitive to the infra-red, a strong solution of copper sulphate was interposed in front of them. No such copper sulphate was used by either Hartmann or Steinmann. It is possible that the discrepancies depend on very small contamination of the light with infra-red.

Due to the kindness of Dr. D. D. Van Slyke and of Dr. F. W. J. Roughton, it was made possible to determine by both gasometric and the present methods the carbon monoxide hemoglobin concentration of the same samples of a series of several bloods. Of one of these samples the oxygen capacity was also determined gasometrically in order to check our ϵ value of oxyhemoglobin in the green line. In this blood the oxygen capacity determined photometrically was practically identical (0.3 per cent higher) with the value obtained gasometrically when both the oxygen and carbon monoxide content were included in the latter determination. Assuming the photometric determination of the hemoglobin concentrations as correct for the other bloods, the *absolute* carbon monoxide hemoglobin saturations of three of the samples were found gasometrically to be: *a*, 8.3 per cent; *b*, 15.4 per cent, and *c*, 10.2 per cent. Photometrically, the *differences* between the control and experimental bloods were estimated as : *a*, 7.8 per cent; *b*, 15.2 per cent, and *c*, 10.1 per cent. The control blood for samples *a* and *b* were 0.8 per cent, for sample *c* 1.4 per cent saturated with carbon monoxide. The differences between the control and experimental bloods appear to be exaggerated by the photometric method. The values of the differences are almost identical with the absolute saturations observed gasometrically.

The effect of temperature. The temperature of the room in which the bloods are read has a significant effect on the ratios of both oxyhemoglobin and carbon monoxide hemoglobin, with the consequence that the higher the room temperature the lower appear to be the percentages of carbon monoxide hemoglobin. This is apparently due to shifts in the optical ratios of both oxyhemoglobin and carbon monoxide hemoglobin. In only a few of the data here reported has any attempt been made to correct for such errors. Without any correction the error introduced by an increase in the room temperature of 4°C was of such an order that a 8.5 per cent saturation of hemoglobin with carbon monoxide might be estimated as 8.0 per cent. During the winter when the room temperatures varied but little the errors were small; we do not believe that such errors have vitiated our conclusions. Estimations of hemoglobin concentrations were not affected significantly (table 4).

[5] The oxyhemoglobin solution was prepared by oxygenating for an hour approximately 4 ml. of the diluted hemolysed blood in a 200 ml. tonometer which was rotated in an electric refrigerator.

This temperature effect appears to be due in part to a temperature coefficient of the "dark current" of the caesium oxide photocells, and should be looked for whenever such photocells are used. This effect may be observed by testing neutral glasses at different temperatures. The maximum sensitivity of the RCA 917 and 919 photocells is around 775 mμ; that of the photocells used by Hartmann (15) between 600 and 700 mμ. Such temperature effects may have existed in his system, but if present were unlikely to have been as great. The newly adopted RCA 929 photocells have a maximum sensitivity at around 370 mμ, and have the advantage of being insensitive to infra-red radiation. These photocells do not show any temperature disturbance when tested with neutral glasses except for a temperature coefficient of −0.08 per cent per

TABLE 4

*Effect of temperature upon hemoglobin concentration as determined from E_{546}**

TEMPERATURE	HEMOGLOBIN CONCENTRATION, GRAMS PER 100 CC.			
	Blood sample 1 O_2Hb	Blood sample 2 COHb	Blood sample 3 O_2Hb	Blood sample 4 COHb
	Experiment 1			
°C.				
23.30	16.55	15.62		
25.35			16.12	15.87
25.65	16.55	15.66		
28.50	16.53	15.66		
29.75			16.11	15.86
	Experiment 2			
27.80	16.34			
28.00	16.34			
30.70			16.20	
30.75	16.34		16.21	
31.00			16.19	
31.10	16.35			

* In experiment 1, blood samples 1 and 2 were read in the photometer at the same time, as were also samples 3 and 4. In experiment 2 the values for samples 1 and 3 were obtained independently.

degree centigrade rise in temperature, which coefficient is probably that of the glass itself.

However the results of a single experiment indicate that there is still a temperature factor (though a smaller one) entering into the determinations of carbon monoxide hemoglobin percentages. The difference in the ratios of the optical densities at the two wave lengths for oxyhemoglobin and for carbon monoxide hemoglobin is reduced about 0.6 per cent per 1°C rise of room temperature.

Hemotocrits, Serum Protein. For *hematocrits* it was found convenient to use mechanically drawn capillary tubing (0.5 mm. bore) of pyrex or of soft glass. After use they were discarded. After the blood was drawn into a tube one end of the tube was sealed in an alcohol flame. Usually six or more hematocrit tubes were prepared for each hematocrit determination. These were placed in a small cylindrical glass

holder which had a large drop of mercury in the bottom. The mercury acted as a cushion and prevented the loss of blood from tubes which were incompletely sealed. Table 5 presents several series of hematocrit values, with the averages, medians and the standard deviations from the averages, to demonstrate the consistency obtainable.

The *specific gravity of serum* was measured by the falling drop method of Barbour and Hamilton (21), and the serum protein (grams per cent) calculated by the formula of Weech et al. (22).

General Procedure. In order to maintain the conditions as constant as possible for comparative purposes, as well as to minimize extraneous factors, the subjects, whenever possible, spent the night preceding the experiment in the air-conditioned room in which the determinations were undertaken. The temperature of the room was maintained throughout at approximately 24°C, except in the spring experiments (April–May) when it averaged around 25.5°C. As a routine the subject emptied

TABLE 5

M.E.M. IV/16/40 LYING (CONGESTION USED)	M.E.M. V/7/40	
	Lying	Sitting
per cent red cell volume	*per cent red cell volume*	*per cent red cell volume*
36.9	35.30	36.15
36.4	35.05	36.85
37 0	34.80	37.10
36.7	35.20	36.55
37.2	35.15	36.60
36.6	34.40	36.40
37.0	35.10	37.35
36.8	35.30	
37.1		
36.6		
Average.....36.8 ± 0.26	35.04 ± 0.30	36.71 ± 0.37
Median.....36.85	35.12	36.60

his bladder at 7:00 a.m., following which he dozed or slept for 1½ to 2 hours before the determinations were commenced. For the experiments on the one female subject tested, the subject arrived between 7:30 and 8:00 a.m., following which she dozed until 9:00 o'clock at about which time the measurements were begun. Breakfast was omitted.

In the spring experiments on H, K, L and M, the subjects, following a morning of laboratory or class, came to the air-conditioned room about 1:00 p.m. where they lay down for at least a half-hour before the blood volume measurements were started. Lunch was omitted. These subjects were paired, H with K, and L with M, determinations being made on both members of a pair at the same time. Because of the possibility that chronic exercise might have some influence on the level of blood volume, one member of each pair (K and L), following two control blood volume estimations, undertook several hours of strenuous hand-ball three times each week plus any additional exercise that they could get. The other members of each pair (H and M) acted as controls, avoiding for the first month any exercise except that required

by a normal daily routine (subject H supplemented this occasionally with moderate walking). At the end of this month these two likewise started exercising.

Except for the few instances noted the blood samples were drawn from one of the arm veins near the elbow without stasis. After a control blood sample was obtained, the subject inhaled the carbon monoxide-oxygen mixture for 15 or 20 minutes. The second blood sample was usually obtained before the removal of the mask, or within a

TABLE 6

Basal blood volumes, lying

NO.	DATE	SUBJECT	SEX	AGE	SURFACE AREA	TOTAL CIRCULATING HEMOGLOBIN	TOTAL CIRCULATING BLOOD VOLUME	RED CELL VOLUME	PLASMA VOLUME
					sq.m.	*gms./sq.m.*	*liters/sq.m.*	*liters/sq.m.*	*liters/sq.m.*
1	X/31/39	H	M	21	1.82	446	3.095	1.327	1.758
2*	XI/ 8/39	H	M	21	1.82	458	3.084	1.345	1.739
3	XI/15/39	H	M	21	1.82	464	3.315	1.425	1.890
4*	XI/30/39	H	M	21	1.82	509	3.397	1.489	1.908
5*	XII/ 1/39	H	M	21	1.82	449	3.165	1.395	1.770
6*	I/25/40	H	M	21	1.82	480	3.390	1.484	1.906
7	III/ 1/40	H	M	21	1.82	461	3.170	1.434	1.736
8†	IV/ 4/40	H	M	22	1.82	467	3.210	1.458	1.752
9†	IV/13/40	H	M	22	1.82	458	3.192	1.445	1.747
Average						466	3.223	1.422	1.801
10	XII/ 1/39	C	M	29	2.07	514	3.472	1.522	1.950
11	IV/16/40	M.E.M.	F	31	1.34	254	2.139	0.780	1.359
12	IV/30/40	M.E.M.	F	31	1.34	266	2.389	0.877	1.512
13	V/ 7/40	M.E.M.	F	31	1.34	269	2.492	0.874	1.618
14†	IV/ 4/40	K	M	22	2.06	452	2.945	1.382	1.563
15†	IV/13/40	K	M	22	2.06	472	3.000	1.475	1.525
16†	IV/ 6/40	L	M	21	2.02	389	2.420	1.156	1.264
17†	IV/11/40	L	M	21	2.02	367	2.360	1.180	1.180
18†	IV/ 6/40	M	M	21	2.10	431	2.822	1.320	1.502
19†	IV/11/40	M	M	21	2.10	441	2.855	1.332	1.523

† Values included in the data for figures 2 and 3.

Nos. 1–7, in air-conditioned room overnight. Lying basal. Nos. 8–9, following morning of class. No lunch. Lying 1:00–1:30 p.m. No. 10, in air-conditioned room overnight. Lying basal. Nos. 11–13, not in air-conditioned room overnight. Lying basal. Nos. 14–19, following morning of class. No lunch. Lying 1:00–1:30 p.m.

minute following its removal. In the experiments on posture, usually the subject was moved (with as little movement as possible on his part) from one position to the other without breaking the connection of the lung-Sanborn system.

RESULTS. *Basal Blood Volumes.* Table 6 shows blood volume estimations obtained on six subjects while lying on a bed in a basal or semi-basal condition. Usually the blood volume measurements obtained in the

morning were carried out between 9:00 and 10:00 o'clock, in some cases (starred) between 10:00 and 11:00.

Of interest are the results of the 9 experiments on subject H, which were obtained at intervals over a period of five and one-half months, from the last of October to the middle of April, the winter season in Philadelphia. As may be seen from the table, his total hemoglobin, and his cell and plasma volume varied but little. If the data of XI/30/39 are omitted (data obtained simultaneously with his first experience as subject for the dye method), the total spread, taking the lowest values as 100 per cent, were for total hemoglobin, cell and plasma volumes: 7.6 per cent, 11.8 per cent, 9.8 per cent respectively. These data show the reproducibility of results by this method.

In a recent paper Forbes et al. (23) report blood volume estimates (by T 1824) on ten laboratory workers which were obtained in Boston during the winter months. The average blood volume per square meter of surface area of their subjects under these conditions was 3.24 liters. Omitting the data obtained on the female subject, the average blood volume, per square meter, of our subjects was 2.98 liters, or 8 per cent below that of the Boston group. This discrepancy in the absolute levels is of interest, for it is of the order of the difference found between the values of blood volume estimates made simultaneously by congo red and by carbon monoxide (10). We are not in a position to explain this discrepancy; it may be accounted for on the basis of the hematocrit error, errors in our estimates of the absolute levels of percentage carbon monoxide hemoglobin, errors in the chance sampling of a population, or factors unknown.

The variation in the size of the blood volume with different individuals observed by Gibson and Evans (24) using the dye T 1824 is reflected in the data of table 6. The values obtained in April on subjects H, K, L and M (4 students carrying the same program) show marked individual differences. As mentioned earlier in this report, the subjects were paired, H with K and L with M, both members of a pair acting as subjects at the same time, and therefore under the same conditions, and following the same morning routine. Yet the blood volume per square meter of surface area of subject H is greater by 6 per cent to 9 per cent than that of his partner K, and that of subject M greater by 16 per cent to 21 per cent than that of his partner L. The latter two subjects, who show the larger differences, were physically fit, approximately of the same height, weight, and surface area, but differed considerably in build. Obviously these differences cannot be seasonal, nor can they be attributed to the method of determination, for they were observed also by Gibson and Evans (24) and by Forbes et al. (23) using T 1824.

Seasonal Variations. The influence of environmental temperature on the blood volume of man was first recognized by Barcroft et al. (25) in

1922. Since then various workers have recognized seasonal influences on various circulatory reactions (26, literature cited), but until re cntly little attention has been given to seasonal variations in blc od volume. In a recent report, Bazett et al. (26) present data showing seasonal variations in individuals which in some cases were as great as 29 per cent. In the same paper these authors report data obtained in several experiments on subjects living for 10 to 12 days under constant temperature conditions in an air-conditioned room, the temperature of which could be adjusted to imitate the hot summer or the chilly spring weather of Philadelphia. The data show that heat-adapted subjects tend to decrease their blood volumes when exposed for a sufficient period to a continuously cold environment, and conversely, cold-adapted subjects tend to increase their blood volumes when exposed to a continuously hot environment. Under these artificial and acute "seasonal" variations, the shifts in blood volumes ranged from 8 per cent to 32 per cent. More recent data obtained by Forbes et al. (23) show increases of only 1.7 per cent to 10 per cent in the blood volumes (per square meter) of subjects as the result of moving from a cold northern environment to a warm southern climate. The effect of the normal changes in climate has not, however, previously been investigated by the repeated systematic examination of a group of subjects.

In figures 6 and 7 are plotted the results obtained from 6 determinations on each of the 4 subjects, H, K, L and M, during the transition from a cold winter to a mild spring. In figure 6 are plotted the daily mean outdoor temperatures (obtained from the weather bureau), and the averages of the values for hemoglobin concentrations, serum protein concentrations, hematocrit and total blood volume (per square meter of body surface). These average values are the means of single experiments on each of the four subjects, and since the subjects were not all tested on the same day, the mean values represent the averages for the subjects over some period of time (solid lines). In addition, the individual curves for total blood volume (per square meter) are given. In figure 7 are presented the curves for the average (calculated as above) total circulating hemoglobin and for the average total blood, cell and plasma volumes, all calculated as per square meter of surface area.

The average *total blood volume* shows a consistent and marked increase which parallels the general rise of the outdoor temperature. In comparison with the individual blood volume curves, this curve of the averages minimizes the changes but tends to iron out the individual variations due to uncontrollable factors, giving, thereby, a truer picture of the general trend. However the individual curves follow the average curve in their general contour, and this was found to be true of the other data. A feature of the individual curves is the tendency for the values for the two subjects measured on the same day to go in the same direction. On the whole

these shifts which are common to both members of a pair follow the temperature changes preceding the day on which the determinations were made. Evidence of a definite lag in the response to a temperature change

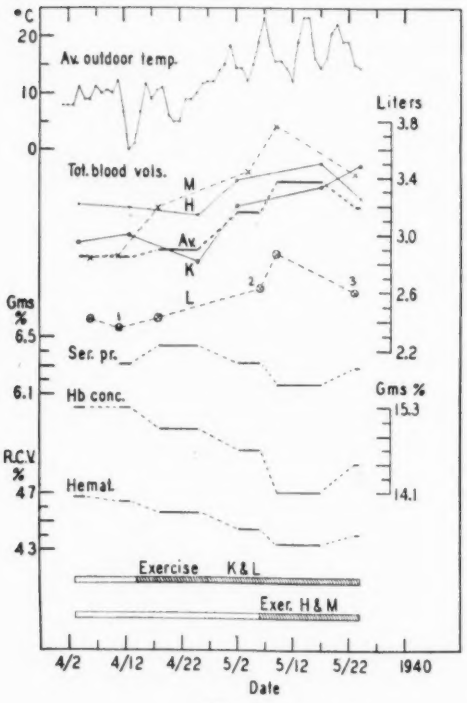

Fig. 6

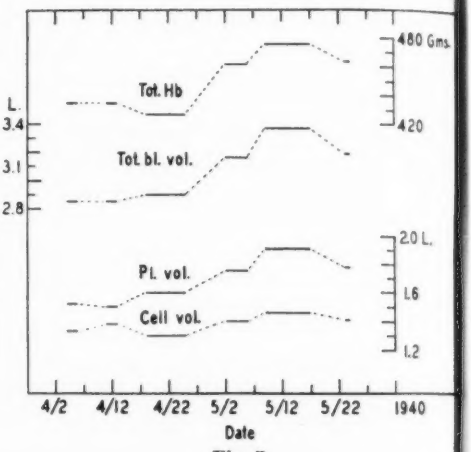

Fig. 7

Fig. 6. Graph showing the relation of the average outdoor temperature to the average hematocrit ratio (*Hemat*), hemoglobin concentration (*Hb conc.*), serum protein concentration (*Ser pr.*) and total blood volume (*Tot. blood vols. Av.*) and to the individual total blood volumes (M., H., K., and L.).

Footnotes, subject L: 1. Vomited day previous to determination. Felt dehydrated. 2. Peripheral veins notably enlarged. Two days previously veins appeared engorged. 3. Marked diuresis previous to determinations.

Fig. 7. Graphs of the average values for total hemoglobin (*Tot. Hb.*), total blood volume (*Tot. bl. vol.*), plasma volume (*Pl. vol.*) and red cell volume (*cell vol.*).

is seen in the curve of subject K, but is not evident in the curves of the other three subjects.

The curves for *hemoglobin concentration, hematocrit ratio and serum protein* are mirror images of the curve of the average blood volume, indicating a definite dilution of the blood concomitant with the increase in

the blood volume. It might be argued that the changes in blood volume and in hemoglobin concentration were artifacts depending on the admitted temperature error of the photometer, or on some other error inherent in the method. Against such arguments are the mirror changes in the hematocrit ratios and the serum protein, determinations which were carried out completely independently. As the temperature of the room in which the bloods were read did not vary by more than 2°C, the maximum error due to the effect of temperature on the photometer would be 5 per cent.

The changes in *total hemoglobin* and in *cell and plasma volumes* (fig. 7) parallel closely the changes in the total blood volume. Obviously, the changes in the estimated total blood volume must be the sums of the changes in the cell and plasma volumes. The plasma volume shows an earlier and a greater increase than the cell volume. These data substantiate those of Bazett et al. (26) who observed that cold-acclimatized subjects when exposed to prolonged heat in the air-conditioned room increased their blood volumes initially by increasing their plasma volumes (sometimes faster than they could supply plasma proteins, resulting in a temporary fall in the serum protein concentration).

The *average changes* observed in the present experiments for total hemoglobin, total blood volume, cell volume and plasma volume were respectively: + 18.2 per cent, +22.6 per cent, +17.5 per cent and +31.3 per cent. These averages are based on the greatest changes observed in each of the four subjects individually, and consequently are higher than the averages calculated from the data used for figures 6 and 7. The *minimal and maximal changes* observed individually were, in the same order as given above: +12.2 per cent to +22.1 per cent, +11.5 per cent to +33.7 per cent, +14.2 per cent to +22.5 per cent, and +13.2 per cent to +45.3 per cent.

The variations in the extent of the changes in blood volume seen in the different individuals show some correlation with the degree of the changes in hemoglobin concentrations, hematocrit ratios and serum protein percentages. Table 7 gives the greatest changes observed in total blood, plasma and cell volumes, hemoglobin concentration, hematocrit ratio (per cent red cell volume) and serum protein (grams per cent) of each of the four subjects. Of the extremes, subject H who had the smallest increase in total blood volume, showed the least decrease in plasma protein; while subject M, who had the largest increase in total blood volume, showed the largest decrease in serum protein. These data likewise correlate with the increase in plasma volumes, subject H showing the smallest increase (+13.2 per cent), subject M the largest increase (+45.3 per cent). As suggested by the figures (table 7) the changes in concentrations (serum protein, hemoglobin and hematocrit) depend on the difference in the rates

with which new equilibria are established. Fluid is increased more rapidly than is protein, and serum protein, in its turn, more rapidly than are red cells.

As may be seen from the individual curves in figure 6, *exercise* had no apparent effect on the total blood volumes. This was true also of the other data. It is quite likely that the exercise was not sufficiently strenuous nor carried over a sufficiently long period to induce measurable changes.

The Effect of Posture. There are comparatively few data in the literature concerning direct measurements of the effect of posture on blood volume. Thompson et al. (27) using the dye method of Keith, Rowntree and Geraghty, obtained a reduction in total blood volume and in plasma volume of an average of 12 per cent in subjects as the result of a shift from the recumbent to the standing position. The average change in the red cell volume was negligible. Waterfield (28), employing the carbon monoxide method of Chang and Harrop, reported that the results of a similar postural

TABLE 7

Maximal changes observed in each of the four subjects

SUBJECT	TOTAL BLOOD VOLUME	RED CELL VOLUME	PLASMA VOLUME	HEMOGLOBIN CONCENTRATION (GRAMS PER 100 ML.)	HEMATOCRIT (PER CENT RED CELL VOLUME)	SERUM PROTEIN (GRAMS PER CENT)
	per cent change	*per cent change*	*per cent change*	*per cent change*	*per cent change*	*per cent change*
H	+11.5	+14.2	+13.2	−4.1	−5.3	−3.3
K	+23.4	+22.5	+24.2	−9.6	−9.7	−5.2
L	+21.6	+12.0	+42.4	−14.3	−13.0	−5.9
M	+33.7	+20.4	+45.3	−11.0	−9.3	−6.3

change caused in his subjects an average reduction in blood volume of 15 per cent, which reduction was associated not only with a decrease in the plasma volume, but also with a fall in the red cell volume of 4 per cent.

We have obtained some few data on blood volume changes associated with a shift from the recumbent to the sitting position. These data are presented in table 8. As would be expected, the changes observed are not as clear cut nor as great as those obtained by a shift from the recumbent to the standing position. In the latter case there is a marked loss of plasma into the legs and feet as the result of the increase in blood pressure due to gravity. Our data indicate a similar but smaller gravity factor acting to decrease the plasma volume, with the result that the blood volume decreases in spite of an apparent increase in the red cell volume. The increase in the red cell volume is difficult to explain, and might be considered as due to a hematocrit error except for the fact that in the experiments on M. E. M. this change was accompanied by an increase in the total hemoglobin. Another possible explanation is that, associated with the

unavoidable movement when changing from the recumbent to the sitting position, some of the carbon monoxide is lost from the hemoglobin to the muscles. It is unlikely to be a "mixing" error (see below) for care was taken that the carbon monoxide was as well mixed as possible while the subject was still lying. In conclusion one may state that as a result of a change from a lying to a sitting position, there is a decrease in the plasma volume which decrease is less than that seen upon standing. One cannot, however, on the basis of the present data, draw any conclusions as to the changes which may occur in the red cell volume.

The Question of Mixing. In either the dye or carbon monoxide methods the question of uniformity of mixing is of prime importance. For subjects

TABLE 8

Blood volume and posture

NO.	DATE	SUBJECT	TOTAL Hb		TOTAL BL. V.		RED CELL V.		PLASMA V.		Hb CONCENTRATION		HEMATOCRIT		SERUM PROTEIN	
			Per m²	Per cent change	Per m²	Per cent change	Per m²	Per cent change	Per m²	Per cent change	Per m²	Per cent change	Red cell volume	Per cent change	Per 100 ml	Per cent change
			grams		*liters*		*liters*		*liters*		*grams*		*per cent*		*grams*	
1	III/ 1/40	H	461		3.17		1.44		1.73		14.53		45.25			
2			463	+0.4	3.08	−2.8	1.46	+1.4	1.62	−6.3	15.02	+3.4	47.25	+4.4		
3	IV/30/40	MEM	266		2.39		0.88		1.51		11.15		36.7		6.10	
4			275	+3.4	2.39	0.00	0.89	+1.1	1.50	−0.66	11.50	+3.1	37.35	+1.8	6.25	+2.5
5	V/ 7/40	MEM	269		2.49		0.87		1.62		10.79		35.04		5.88	
6			272	+1.3	2.42	−2.9	0.90	+2.8	1.52	−6.0	11.25	+4.3	36.71	+4.8	6.15	+4.6
Average				+1.7		−1.9		+1.8		−4.3		+3.6		+3.7		+3.6

No. 1, lying basal. No. 2, after 1 hour of sitting. Second dose CO for independent blood volume determinations. No. 3, lying basal. No. 4, after 11 minutes sitting. Rebreathing apparatus not disconnected between the lying and sitting determinations. No. 5, lying basal. No. 6, after 10 minutes, sitting. Rebreathing apparatus not disconnected between the lying and sitting determinations.

in the recumbent position the usage of the die away curve (4, 5) in the dye method appears to meet adequately this problem, while according to Chang and Harrop (9) 17 or more minutes of rebreathing appears sufficient for uniform distribution of carbon monoxide in the blood. On the other hand there is evidence that in seated subjects uniform distribution of either dye or carbon monoxide may not occur within the periods believed to be sufficient for the recumbent position. This is due, probably, to the slower peripheral circulation. Lindhard (29) was unable to obtain adequate mixing of vital red in the plasma of seated subjects unless they raised their arms above their heads and walked around. Steinmann (19), who compared the percentages of carbon monoxide hemoglobin of

several blood samples drawn simultaneously from various peripheral vessels, was unable to find uniform mixing in some of his seated subjects even though they rebreathed the carbon monoxide for an hour. Conse-

TABLE 9

*Mixing errors**

EXP. NO.	DATE	SUBJECT	BLOOD SAMPLE	REBREATHING TIME	COHb	TOTAL Hb	TOTAL BLOOD VOLUME	CONDITIONS
				min.	*per cent*	*grams*	*liters*	
1	I/25/40	H	1	19	7.38	876	6.15	Lying
			2	30	7.43	871	6.19	Lying
2	IV/ 4/40	H	1	20	8.33	851	5.85	Lying moved hands, feet between samples
			2	28	8.33	851	5.85	Lying
3	IV/ 4/40	K	1	22	7.08	925	6.02	Lying moved some belly muscles between samples
			2	27	7.03	929	6.07	Lying
4	IV/18/40	L	1	21	10.03	760	4.93	Lying moved hands, feet between samples
			2	33	10.22	746	4.86	Lying
5	V/ 2/40	H	1	20	7.92	879	6.22	Lying lying on side between samples
			2	31	8.01	870	6.07	Lying
6	I/16/40	A	1	22	8.47	792	5.23	Sitting (95 min.)
			2	35	7.70	874	5.91	Lying (13 min.) Blood samples obtained with difficulty 12 min. after rebreathing stopped. Loss of CO during this time would reduce the COHb to about 8.28 per cent†
7	II/26/40	A	1	15	8.35	805	5.25	Sitting (83 min.) moved arms, legs between samples
			2	25	8.03	839	5.41	Sitting (93 min.)
			3	31	7.28	909	5.84	Sitting (99 min.)
			4	42	7.67	879	5.73	Lying (11 min.)

* In all of these experiments the subject remained connected to the rebreathing apparatus even while changing position.

† Calculated from formula $C_t = C_0 e^{-at}$ (Stadie and Martin, J. Clin. Investigation **2**: 77, 1925) where a (av. value exp. obtained by us under basal conditions) = 0.0019.

quently, unless care has been taken to insure thorough mixing, data (by either method) on the blood volumes of seated, and presumably also of standing, subjects must be interpreted with caution.

Table 9 presents data concerning adequate mixing on several of our

subjects. Experiments 1 through 5 are data obtained on subjects in the recumbent position. As Chang and Harrop found, 20 minutes appear to be sufficient for adequate mixing, for even movements of the feet and hands, and in one case some of the belly muscles, do not significantly alter the carbon monoxide hemoglobin percentage. In experiments 4 and 5 the second samples show increases (? error). Experiments 6 and 7, show clearly the possible error that may enter into a sitting blood volume determination: in experiment 6 there was no control over mixing, and the apparent increase in the blood volume as the result of taking the recumbent position may be due to sudden distribution of carbon monoxide among red cells previously unable to obtain their share of the gas. Comparison of experiment 7 with experiment 6 indicates that the apparent increase in blood volume of experiment 6 occurring as the result of such a change in position may be erroneous, for slight movements of the feet and legs between samples 2 and 3 (expt. 7) caused a marked fall in the carbon monoxide hemoglobin concentration, which concentration was then found to increase as the result of taking the recumbent position (sample 4). This increase in the percentage of carbon monoxide hemoglobin occurring as the result of changing from a sitting to a lying position is puzzling; it has been observed consistently in cases where presumably adequate mixing has occurred, as in observations during the disappearance of the gas 5 hours or more after its administration. It may indicate a sudden addition to the circulation of previously trapped cells which had maintained a high carbon monoxide content. Further work is necessary to establish this point, but it suggests that there may be uneven distribution of the gas (? dye also) following initial uniform mixing.

Consequently, in the experiments on sitting versus recumbent blood volumes given in table 8, care was taken to insure as adequate mixing as possible while the subject was still lying (M.E.M.) or sitting (H) by having the subjects move their feet and hands.

Experiments on *acute exercise* require the same type of critical examination. Chang and Harrop (9) found an apparent increase of 1.3 per cent to 7.3 per cent in the blood volumes of subjects as the result of exercise on a stationary bicycle, which they believed could be accounted for on the basis of the loss of carbon monoxide from the blood to the muscle hemoglobin, or possibly in part to a real increase due to the addition of splenic blood. On the other hand, Kaltreider and Meneely (30), on the basis of changes in the die away curves following T 1824 injections, found a decrease in the plasma volumes, and consequently in the total blood volumes, of subjects as the result of acute exercise, which decreases were accompanied, following exhaustive exercise, by increases in the red cell volume due presumably to the addition of cells from the spleen.

We have performed only one experiment on exercise, employing moderate

work (4.419 kgm.-m. in 10 min.) on a bicycle ergometer. The results obtained by the present carbon monoxide method were in general agreement with those found by the dye method (30). There was a reduction in the plasma volume estimated as 1.9 per cent, which reduction was substantiated by increases in the hemoglobin and serum protein concentrations. On the other hand there was a small increase (1.2 per cent) in the total blood volume due to an apparent increase in the total hemoglobin, which increase was within the range found by Chang and Harrop.

Discussion. The present carbon monoxide method for blood volume determinations appears to be a reliable method for determining relative values, although the actual levels may be somewhat low. The data obtained by this method indicate that in any one individual the basal recumbent blood volume level may be maintained remarkably constant, provided that the conditions of his ordinary routine do not undergo sudden or progressive changes. The data also show that the levels of the blood volumes of different individuals may vary greatly, as has been found by dye methods (23, 24), even though those individuals follow approximately the same daily routines and are exposed to the same environmental conditions. The consistency of blood volume levels, however, disappears in the spring during the transition from a prolonged cold winter to warmer, even hot, weather, and the increases seen during such a transition have been found not only by the carbon monoxide method but also by the dye (26). The degree of change appears to depend on the individual. It may possibly be influenced by the level of the blood volume at the start of the warm weather (subject H, a native of a Southern state, consistently had a large blood volume throughout the winter, the largest volume per square meter of body surface during the control periods, and the smallest increase during the spring months).

The basal recumbent blood volume may be shifted temporarily as the result of postural changes; standing (27, 28) and probably sitting cause a decrease, apparently due, for the most part, to the loss of plasma into the tissues of the dependent parts.

It is apparent that mixing errors are inherent in the carbon monoxide method especially when the subject is seated. It is likely that under similar conditions they may be present in the dye method as well. Consequently the results on postural studies must be considered with caution.

SUMMARY

1. A carbon monoxide method, similar to that of Chang and Harrop, for determining the blood volume of human subjects is described. The determinations of the percentage of carbon monoxide hemoglobin following the inhalation of carbon monoxide were made by means of a differential electric photometer.

2. The differential electric photometer is described. With careful handling of the blood and the use of a 30 inch bench the hemoglobin concentration may be determined with a standard deviation of ± 0.34 per cent. A percentage of carbon monoxide hemoglobin of approximately 10 per cent may be determined with a standard deviation of ± 0.82 per cent of that saturation. The determinations of both the hemoglobin concentration and the percentage saturation of the hemoglobin with carbon monoxide may be determined on the same hemolysed blood sample for which only 0.04 to 0.4 ml. of whole blood are required.

We confirm others using a similar photometer, in the finding that the ratio $\Delta D_{546}/\Delta D_{578}$ of oxyhemoglobin varies with bloods from different individuals and with the different samples of blood from the same individual. These variations are correlated with different amounts of carbon monoxide hemoglobin which exist in the bloods of different individuals, e.g., smokers and non-smokers, and with changes in the temperature at which the blood is read.

3. Values for basal blood volumes of recumbent subjects are given, the absolute levels of which may be somewhat low, but which show that under constant conditions any one individual may have a remarkably constant volume (total spread of 10 per cent in nine determinations on one subject over five and one-half months). This constant recumbent level however varies in different individuals (males: max. 3.47 liters, min. 2.39 liters, one female: 2.34 liters per square meter of body surface), and in any one individual may be varied as the result of seasonal variations in environmental temperatures (increase in the spring with warm weather 11.5 to 33.7 per cent, average increase 22.6 per cent), and by change in position.

We wish to take this opportunity to thank Mr. A. H. Chambers for making the estimations of oxygen capacity by Van Slyke's method for the standardization of the constants for the electric photometer, and to thank the students who so kindly acted as our subjects even in the face of pressing work. We are also indebted to the John and Mary R. Markle Foundation for a grant toward the expenses of this work.

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PAPAVERINE HYDROCHLORIDE AND VENTRICULAR FIBRILLATION

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In the course of studies on the effect of various agents on the caliber of the coronary vessels of the isolated fibrillating dog heart preparation (1), it was observed that following the injection of papaverine hydrochloride (Lilly), the ventricular fibrillation was replaced by synergic beating. This phenomenon was investigated further to determine whether or not the conversion to a regular beating could be constantly reproduced.

Conversion to regular ventricular beating occurred with doses ranging from 0.5 to 3 cc. of 1:30 dilution of papaverine hydrochloride in five out of six isolated fibrillating heart preparations; the larger amounts were given in 2 or 3 divided doses. This conversion to regular rhythm occurred even after periods of fibrillation of from 40 to 75 minutes had elapsed before the drug was administered. In this preparation, however, the nourishment of the heart is maintained by perfusion of the coronary vessels under suitable pressure with defibrinated blood at normal body temperature (38°C).

Synergic beating was established in those instances in which the papaverine concentration reaching the heart was between 1:200 to 1:340. The concentration was roughly calculated from the amount and time of injection and the rate of coronary flow. Two injections, as close to this concentration as 1:450 and 1:750, respectively, produced very coarse undulatory movements of the ventricular muscle, but no true contractions. Evidently the ability of the drug to abolish fibrillation is intimately connected with its concentration in the blood reaching the heart. In spite of repeated induction of ventricular fibrillation by stimulation with a faradic current from a Harvard inductorium applied directly to the ventricles, the fibrillation so induced disappeared almost immediately upon removal of the stimulating electrodes and was replaced by regular synergic beats. This occurred as late as 8, 12, 12 and 60 minutes respectively after injection. In one experiment the regular beating continued throughout the faradic stimulation of the ventricles, even when the strength of the current had been increased to the point of burning the myocardium.

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This heart went into complete standstill on removal of this maximum stimulation.

This "antifibrillation" effect of papaverine hydrochloride was next investigated in open-chested, artificially ventilated animals anesthetized with nembutal (25 mgm/kilo) or (in 2 cases) with ether. The pericardium was removed to permit exposure of the heart. Faradic stimulation of the ventricles was produced with a Harvard inductorium; the position of the secondary with respect to the primary coil was noted in centimeters, the secondary being kept parallel to the primary coil; the primary coil was activated by 3 volts. In some animals, after production of ventricular fibrillation, the aorta and the two venae cavae were completely occluded, leaving a heart-lung preparation. Whenever the heart appeared to be too empty because of pooling of blood in the lungs, the clamp on the inferior vena cava was released and blood was massaged from the abdomen to the heart. The stimulating electrodes were lightly applied to the right ventricle near the septum. (No evidence of injury to this region was noted.) Stimulation was given for a prescribed period of time with the middle of the secondary coil 10 cm. from the middle of the primary. This strength of stimulus was reapplied 2 times before the current was increased by moving the secondary coil 1 cm. nearer the primary. This new stimulus was also applied three times and the secondary coil again moved 1 cm. The strength of stimulus and, at times, its duration were thus progressively increased until a ventricular fibrillation was finally produced which did not spontaneously disappear after removal of the stimulating electrodes.

In a series of three dogs the papaverine hydrochloride was injected shortly before the ventricles were fibrillated.

The first animal was anesthetized with ether. Two cubic centimeters of 1:30 solution of the drug were injected into the femoral vein, the circulation being kept intact. In this animal persistent ventricular fibrillation did not occur until the secondary coil was within 4 cm. of the primary. A control animal, who received no papaverine, developed persistent ventricular fibrillation when the secondary coil was 10 cm. from the primary. In both instances the current was applied for 1 second.

The second animal was anesthetized with nembutal. A heart-lung-head preparation was made by occluding only the blood supply to and from the lower part of the animal. Six cubic centimeters of 1:120 papaverine hydrochloride were injected directly into the left ventricular cavity. Persistent ventricular fibrillation did not occur until the secondary coil was 7 cm. from the primary. In a control heart-lung-head preparation in which no papaverine was given, persistent ventricular fibrillation occurred following stimulation with the secondary coil 9 cm. from the primary. Again, the duration of stimulation in both cases was 1 second.

In the third animal of this series, anesthetized with nembutal, the circulation was kept intact. Four cubic centimeters of a 1:100 solution of papaverine hydrochloride were injected into the left ventricular cavity. Two applications of faradic stimulation of 2.2 and 2.8 seconds' duration, respectively, with the secondary 9 cm. from the primary caused only temporary ventricular fibrillation with spontaneous restoration of synergic beating. The third application of this strength stimulus for 1.8 second resulted in ventricular fibrillation. After the fibrillation had lasted for more than a minute in this animal, continuous, rapid, manual massage of the ventricles was given in order to insure some coronary circulation, and 2 cc. more of 1:100 papaverine was injected into the left ventricular cavity. After 20 minutes the ventricles began to beat regularly.[2]

Then once again the ventricles were fibrillated with a faradic current of similar strength and duration to that originally producing fibrillation. Massage was immediately instituted and 2 minutes later regular synergic contraction had reappeared.

In another series of three animals the hearts were fibrillated by faradic currents of 1.6, 0.8 and 1.6 seconds' duration, the secondary coil being 10 cm. from the primary. In the first two fibrillating hearts massage *alone* for 38 and 20 minutes, respectively, was ineffective in restoring synergic contractions, even though the massage was adequate to remove the cyanosis and to restore the normal pink color of the heart. Papaverine hydrochloride was then injected into the left ventricular cavity. In the third heart the papaverine was injected soon after the fibrillation was established.

In the first heart, in which 1.5 cc. of 1:100 papaverine hydrochloride were injected 38 minutes after ventricular fibrillation had been induced, the massage, continued for another 40 minutes, resulted in the coarsening of the fibrillatory waves with thick rings passing over the ventricles from apex to base, but failed to restore a synergic beat.

In the second heart, in which 3 cc of 1:100 papaverine had been injected 20 minutes after the onset of ventricular fibrillation, normal beating was

[2] Evidence of the arrested circulation appeared soon after ventricular fibrillation, in the form of cardiac cyanosis and dilatation. Brisk and rapid manual massage (about 100/min.) was found to be effective in restoring at least some circulation, since with it the heart progressively became more pink and smaller in size. Slower massage was not as effective. Care was taken to avoid compression of the circumflex and left anterior descending coronary vessels in order to permit complete recovery of the heart.

Preceding its disappearance, the fibrillation gradually became coarser and more vigorous, individual waves travelling in increasingly greater sweeps across the heart. After this stage the ventricles became "knotty" in appearance, little movement being seen or felt and then immediately before the synergic beat became established the ventricles momentarily showed no activity at all, seeming to be in a systolic standstill.

restored after 22 minutes of massage. In the third heart in which the same amount of papaverine was injected soon after the fibrillation began, synergic beating was restored after 10 minutes of massage.

Attempts were made to refibrillate the ventricles in these last two hearts after the papaverine had caused a return of synergic beating. In the

TABLE 1

Effect of papaverine hydrochloride on the threshold to induced ventricular fibrillation

DOG NO.	ANESTHESIA	DISTANCE OF 2ND COIL FROM PRIMARY	DURATION OF STIMULATION	NUMBER OF TIMES STIMULUS OF THIS STRENGTH APPLIED	PREPARATION	DOSAGE OF PAPAVERINE HYDROCHLORIDE
			Without papaverine			
		cm.	*sec.*			
1	Ether	10	<1	1	Intact circulation	
3	Nembutal	9	<1	2	Heart-lung-head	
5	Nembutal	10	1.6	1	Intact circulation	
7	Nembutal	10	0.8	1	Intact circulation	
8	Nembutal	10	1.6	2	Intact circulation	
			With papaverine*			
2	Ether	4	<1	1	Intact circulation	2 cc. of 1:30 intravenously
4	Nembutal	7	<1	2	Heart-lung-head	6 cc. of 1:120 intracardiac†
6	Nembutal	9	1.8	3‡	Intact circulation	6 cc. of 1:100 intracardiac†
7	Nembutal	5	2.0	1	Heart-lung	3 cc. of 1:100 intracardiac†
8	Nembutal	2	3.2§	1	Heart-lung	3 cc. of 1:100 intracardiac†

* Compare dogs 7 and 8 before and after papaverine.

† Intracardiac injections were into the left ventricular cavity.

‡ Two previous stimulations at this strength for 2.2 and 2.8 seconds; latter lead to fibrillation of 30 seconds' duration, recovered from without massage. Third stimulation applied 1½ minutes after 2nd.

§ This was really ventricular flutter lasting for 30 seconds with spontaneous recovery.

first of these, faradic stimulation for 5 seconds with the secondary coil at 5 cm. caused fibrillation lasting 15 seconds. The ventricles then spontaneously recovered even though no massage was applied. A second faradic stimulation of the same strength and of 2 seconds' duration caused only a momentary fibrillation. A third stimulation of similar strength for 5 seconds led to persistent ventricular fibrillation.

After restoration of synergic beating with papaverine in the second heart, faradic stimulation for 9 seconds with the secondary coil at 10 cm. failed to produce fibrillation; the heart continued to beat synergically, though more rapidly throughout the stimulation. This phenomenon was observed during each of the 5 to 7 second periods of stimulation given thereafter as the secondary coil was made to approach the primary. However, with increasing strength of stimulus, the rate of contraction during stimulation periods increased until the ventricular movements finally became flutter-like when the secondary coil was 5 cm. or less from the primary. When the secondary coil was 2 cm. from the primary, stimulation of the heart for 3 seconds produced flutter-like contractions of the heart which continued for 30 seconds and then without massage disappeared to be replaced by synergic contractions.

The table on page 158 summarizes our experience with the threshold to faradically induced ventricular fibrillation in these animals.

Discussion. Papaverine is an opium alkaloid with low toxicity. The solution we used is dissolved in water and has no apparent impurities. Some 25 years ago Macht (2) in studying the actions of this drug found that it slowed the rate of the mammalian heart, increased its vigour and enhanced coronary flow, in addition to its later better known peripheral vascular actions. These cardiac effects appeared to be exerted directly on the heart. We have found that papaverine is a powerful and persistent direct coronary vessel dilator (3).

Although spontaneous recovery from ventricular fibrillation has been reported in man, e.g., Schwartz and Jezer (4) and Gertz, Kaplan, Kaplan and Weinstein (5), this is not of common occurrence and the known therapy is usually ineffectual. Our results suggest that papaverine hydrochloride may be useful as a prophylaxis of this condition and perhaps as a therapeutic measure when accompanied by massage of the heart.

The marked coronary dilating action of papaverine and its ability to prevent and abolish ventricular fibrillation may explain the benefits to be derived from the use of the drug in pulmonary embolism and in coronary disease and angina pectoris. They probably explain the beneficial results reported recently by McEachern, Smith and Manning (16) in dogs following sudden coronary closure.

At present one can only speculate upon the mode of action of papaverine. The slowing and coarsening of the fibrillation seen before its abolition suggest that the drug 1, slows the rate at which impulses are transmitted in the ventricles; and 2, lengthens the refractory period of the myocardium. In view of the recent observations of Wiggers and Wegria (7) on the low threshold to fibrillation existing during the vulnerable period of the cardiac cycle, the greater difficulty in producing ventricular fibrillation after papaverine suggests that the drug raises the threshold of vulnerability.

SUMMARY

1. Papaverine hydrochloride, in addition to being a powerful coronary vasodilator, also considerably decreases the ease with which ventricular fibrillation is induced in the dog by faradic stimulation.

2. In its presence, vigorous massage of the heart restores a regular synergic beating to ventricles which have been in fibrillation.

3. These actions of the drug permit its application therapeutically and prophylactically, not only where marked protracted coronary dilatation is desirable but also in conditions which are apt to lead to ventricular fibrillation.

We are indebted to Dr. K. K. Chen of Eli Lilly & Company for supplying us with the papaverine hydrochloride.

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THE EFFECTS OF TRAINING AND OF GELATIN UPON CERTAIN FACTORS WHICH LIMIT MUSCULAR WORK

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Since glycine has been reported by some authors to be beneficial in the treatment of certain muscular diseases several papers have appeared concerning its effect upon muscular fatigue in normal people. Boothby (1934) and Wilder (1934) have reported that glycine will decrease fatigability in normal men. While glycine is a common constituent of protein foods it is usually present only in low concentrations. Gelatin, which is 25 per cent glycine, may therefore produce different effects from other proteins. Gelatin has been reported by Ray, Johnson and Taylor (1939) and by Kaczmarek (1940) to increase by more than 200 per cent the work output of men working to exhaustion on a bicycle ergometer. Ray et al. found that the performance of women in the bicycle experiments was not affected by gelatin and their women subjects improved only slightly with training. Their data show an unusually great difference in the power of men and women subjects. Kaczmarek (1940) reported that 12 girls improved 500 per cent in 4 weeks under the influence of 43 grams of gelatin per day. These authors suggest that the effect of gelatin is related to a creatinogenic action of the glycine. Hellebrandt, Rork and Brogdon (1940) carried out experiments similar to those of Ray et al. using adult women as subjects. The effects of training were great but those of gelatin were negligible. The work output of their female subjects exceeded that of the men used by Ray and associates.

This work was undertaken in view of these contrasting reports. In addition to measurements of performance in exhausting work we have incorporated a number of objective tests to meet the contention that psychological factors may have influenced performance. If gelatin influences the capacity for intense work of short duration it must do so by increasing the rate at which O_2 can be supplied to the tissues, or the amount of energy from anaerobic sources available for the work, or the efficiency with which the work is done. Tests were designed to measure all of these functions.

Nine non-athletic college men, ages 18 to 22, were used as subjects in the

study. Since the work consisted of walking and running on a motor driven treadmill and of timed races on the track, the subjects were trained by a regular and carefully supervised running program which continued for 26 weeks. A description of the subjects and the conditions of training have been previously reported (Robinson and Harmon, 1941). The 9 men were divided into two groups. The 6 men of group I took 60 grams of gelatin each day from the 9th through the 15th week of the training period and the 3 men of group II took the same amount of gelatin from the 15th through the 21st week. The gelatin was suspended in water and given at meal times under the supervision of an assistant in our laboratory. It was expected that any effects produced by gelatin would be revealed in the curves of performance for the gelatin and non-gelatin groups.

Observations in the laboratory were made on the men before training started and at regular intervals during the training period. *A*. Efficiency was tested in two different grades of aerobic work on a motor driven treadmill: 1. A standard 15-minute walk at 5.6 km. per hour on a grade of 8.6 per cent, expired air for measuring O_2 consumption being collected from the 8th through the 14th minute. 2. A 10-minute run on the level at a moderate pace which was 12.9 km. per hour for 7 of the men and 14 km. for the two best runners; metabolism was measured from expired air collected from the 6th through the 10th minute of the run. *B*. The maximal capacity of the men for supplying O_2 to the tissues and their ability to utilize anaerobic energy in severe work were tested on the treadmill by exhausting runs of 3 to 5 minutes' duration. During the training period as a man became able to complete 5 minutes of the exhausting run the grade or speed or both were increased for him in the next test in an attempt to keep the work just severe enough to exhaust him in 4 to 5 minutes, his goal being to complete the 5 minutes. Metabolism was measured from expired air collected in a 500 l. gasometer during the run, records of respiratory volume being made for each minute. Samples for analysis were collected from a mixing chamber in the expiratory tube concurrently. Air expired during the first 15 minutes of recovery was collected in Douglas bags. Oxygen used in excess of the resting level during this time gave a direct measure of a considerable fraction of the O_2 debt and probably included all of the "alactacid oxygen debt". The extent to which the lactic acid mechanism was utilized was measured by lactic acid concentration in venous blood drawn 5 minutes after the end of work. This gives an objective measure of the state of exhaustion of the subject. *C*. Total nitrogen and creatinine excretions were determined before training started, immediately before gelatin was started, just before the ends of the gelatin periods, and finally at the end of the training period. Each value reported represents the average of three consecutive 24-hour urine samples. Basal metabolism was also determined at these times by collecting expired air in a spirometer.

Creatinine was analyzed by the method of Folin (1914) modified for the photoelectric colorimeter, total nitrogen in urine by the Kjeldahl method, and gas samples were analyzed on the Haldane apparatus. The other analytical procedures used have been described by Robinson and Harmon (1941).

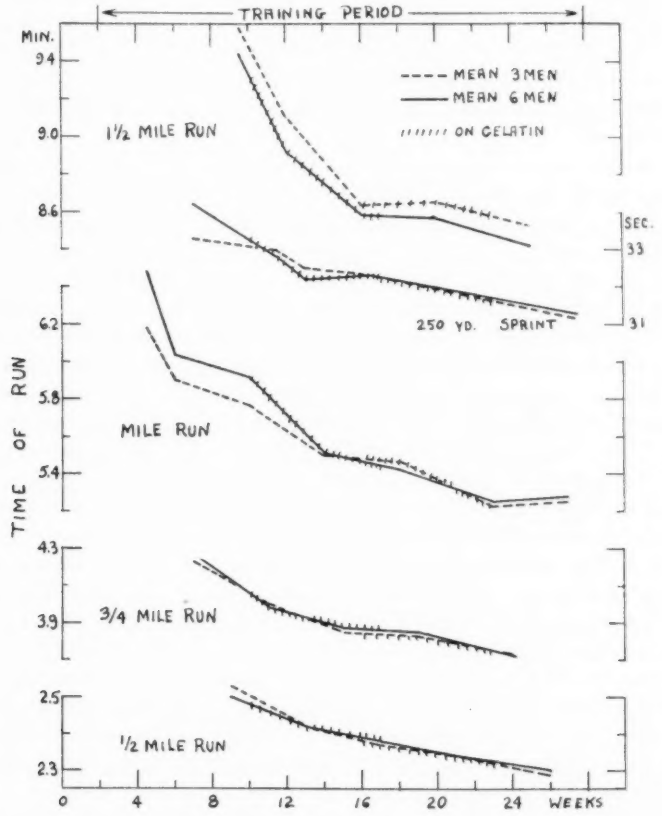


Fig. 1. Times of the weekly races on the track. The group averages in the various distances show no effect of gelatin but consistent improvement with training.

RESULTS. *Performance in races* on the track served to measure changes in the capacities of the men for severe work. Rivalries between men of about equal ability were encouraged to motivate performance and they became very keen in most cases. Figure 1 gives the average results of the weekly races for the two groups and shows the intervals at which the various distances were run. Improvement with training in all distances was consistent. In most instances the men ran the races in a group and thus

were exposed in equal measure to adverse weather conditions, etc.—this probably accounts in part for similarity in the variations of the means of the two groups. For instance the final times in the mile run were no better than in the preceding trial at this distance because of a high wind which slowed up performance on the final day. It is evident from the mean values plotted in figure 1 that gelatin did not influence the rate of improvement of the men. The report that it increases the anaerobic release of energy indicates that performance in the shorter runs should have been improved and yet we find no differences there in favor of the gelatin periods. Our results are at complete variance with those of Ray and associates and of Kaczmarek.

Metabolism in exhausting work. In the exhausting runs of 4 to 5 minutes' duration the O_2 debt and O_2 consumption during work were of about equal importance to the men as sources of energy. We have no reason to believe that gelatin would affect a man's capacity for O_2 consumption but since that is an important limiting factor in work of this type and might be influenced by training we measured it in the exhausting runs on the treadmill. In these experiments we found that men approached their maximal rates of O_2 consumption in the third minute and made only slight increases in the 4th and 5th minutes of work. Figure 2 shows the average maximal O_2 intake of the two groups of subjects in relation to the training and gelatin periods. At first the men, under the influence of training, made rapid improvement in their ability to consume O_2 and in general continued to improve until the end. The total increase averaged about 16 per cent. Group I showed no change in the rate of improvement which can be attributed to gelatin and finally attained its highest average 9 weeks after gelatin was stopped. Group II composed of only 3 men showed greater variability of the mean values than group I but ultimately attained its highest average value 5 weeks after gelatin was stopped. These men were in a slump at the time they started gelatin so we cannot attach any significance to their rapid rise in the latter part of the gelatin period particularly since the 6 men of group I had a decline during the last part of their gelatin period. We conclude that improvement in the supply and utilization of O_2 was unaffected by gelatin.

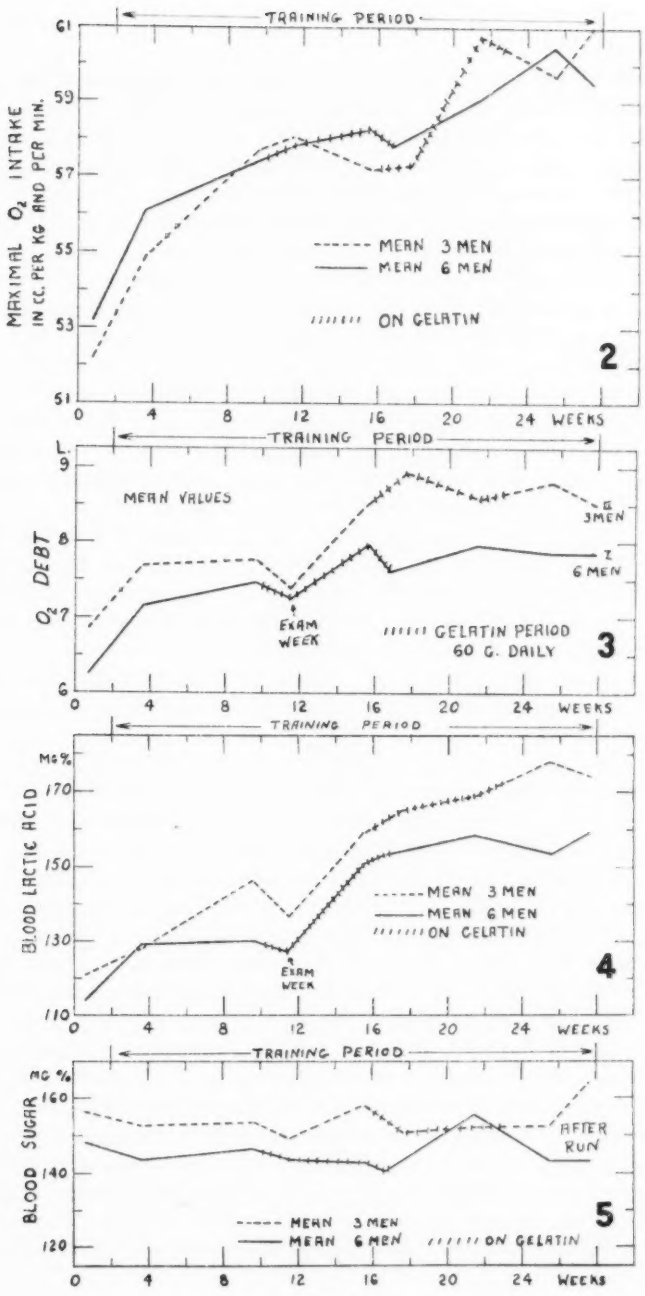
If gelatin has a creatinogenic action it might possibly exert a beneficial

Fig. 2. Group averages of maximal O_2 intake during the exhausting runs on the treadmill.

Fig. 3. Excess O_2 intake above the resting level (O_2 debt) during the first 15 minutes of recovery after running to exhaustion on the treadmill.

Fig. 4. Group averages of blood lactic acid 5 minutes after running to exhaustion on the treadmill.

Fig. 5. Group averages of blood sugar 5 minutes after running to exhaustion on the treadmill.


TRAINING PERIOD
MAXIMAL O_2 INTAKE IN CC. PER KG AND PER MIN.
61
59
57
55
53
51
MEAN 3 MEN
MEAN 6 MEN
ON GELATIN
2
0
4
8
12
16
20
24 WEEKS
TRAINING PERIOD
O_2 DEBT
L.
9
8
7
6
MEAN VALUES
3 MEN
6 MEN
EXAM WEEK
GELATIN PERIOD
60 G. DAILY
3
0
4
8
12
16
20
24 WEEKS
TRAINING PERIOD
BLOOD LACTIC ACID
MG%
170
150
130
110
MEAN 3 MEN
MEAN 6 MEN
ON GELATIN
EXAM WEEK
4
0
4
8
12
16
20
24 WEEKS
TRAINING PERIOD
BLOOD SUGAR
MG %
160
140
120
AFTER RUN
MEAN 3 MEN
MEAN 6 MEN
ON GELATIN
5
0
4
8
12
16
20
24 WEEKS

Figs. 2–5

influence in this type of work by increasing the "alactacid oxygen debt" which, according to Margaria, Edwards and Dill (1933), is repaid during the first few minutes of recovery and is probably associated with the breakdown and resynthesis of phosphocreatin and perhaps with other anaerobic processes besides the lactic acid mechanism. Thus our measurement of excess O_2 intake above the resting level during the first 15 minutes of recovery would include all of the alactacid oxygen debt and a good part of that involving the lactic acid mechanism. Figure 3 shows that the magnitude of this excess O_2 consumption in early recovery increased during the first two-thirds of the training period with no changes which could be attributed to gelatin. The increases and variations of the means correspond closely with changes of lactic acid in blood drawn 5 minutes after the runs (fig. 4). This is to be expected since the major part of the maximal O_2 debt is related to the lactic acid mechanism. Five minutes after exhausting work of short duration lactic acid has become uniformly distributed between tissues and blood according to evidence previously cited (Robinson and Harmon, 1941). It is evident from figure 4 that gelatin had no effect on the ability of the men to utilize the lactic acid mechanism for contracting an O_2 debt.

The elevation of blood sugar above basal after the exhausting runs on the treadmill was not affected by gelatin (fig. 5).

Efficiency in submaximal work. A study of the efficiency in running was made by measuring the O_2 consumption during the last 4 minutes of a moderate run of 10 minutes' duration in which the men could approach a steady state and supply the energy aerobically (table 1). In the test before training was started it is probable that most of the men were not doing all the work aerobically because the average apparent R.Q. in the metabolism period was 1.03 indicating that they were producing some lactic acid and displacing CO_2 from the alkaline reserve (table 1). The blood lactates 5 minutes after work averaged 77 and 69 mgm. per cent in the two groups at this time and the rates of O_2 consumption were about 90 per cent of the maximal values which the men could attain. After 4 weeks of training only 2 of the men had R.Q. values above unity and the lactate averages had dropped to 56 and 63 respectively. Rates of O_2 consumption in the tests after this probably measured the total energy requirement for the run. Even though the initial O_2 consumption may have been somewhat lower than the actual O_2 requirement for the run the 9 men made an average improvement of about 8 per cent by the 11th week of training. One of the individuals who was moderately skilled at first made no improvement on this test. Both groups showed a small loss of efficiency in the last test from a plateau which they had attained previously. The data in table 1 show that gelatin had no effect on the efficiency in this test.

Since it has been claimed that fatigue in carrying out daily duties is greatly affected by the use of gelatin we measured the mechanical efficiency

TABLE 1

Mean values of metabolic adaptations to the walk at 5.6 km. per hour on a grade of 8.6 per cent and to the 10-minute run on the level

	SUBJECTS	BEFORE TRAINING	AFTER 4 WEEKS TRAINING	AFTER 11 WEEKS TRAINING	AFTER 18-21 WEEKS TRAINING	AFTER 25-26 WEEKS TRAINING
Mechanical efficiency† in grade walking	6 men	17.0	17.3	17.5*	17.2	17.5
	3 men	16.0	18.2	17.8	17.5*	18.2
O_2 consumption in 10-minute run, cc. per kgm. per min.	6 men	48.8	46.9	44.3*	43.7	44.5
	3 men	46.9	45.5	43.9	45.2*	46.1
R.Q. in 10-minute run	6 men	1.03	0.97	0.95*	0.94	0.94
	3 men	1.03	1.00	1.00	0.97*	0.96
Blood lactate in 10-minute run, mgm. per cent	6 men	76.6	55.7	44.6*	30.7	31.4
	3 men	68.5	62.7	53.4	45.5*	43.0

* Values recorded during the period of gelatin feeding.

† M.E. $= \dfrac{\text{Work} \times 100}{(\text{Total energy}) - (\text{Basal energy})}$

Work = grade lift in walking on the treadmill expressed as Cal.

The total and basal energy exchanges were calculated in Cal. from the O_2 consumption.

TABLE 2

Mean values of basal metabolism and the daily urinary excretion of total nitrogen and creatinine

	SUBJECTS	BEFORE TRAINING	AFTER 8 WEEKS TRAINING	AFTER 14-15 WEEKS TRAINING	AFTER 20-21 WEEKS TRAINING	AFTER 25-26 WEEKS TRAINING
Basal metabolic rate—Cal. per M^2. per hr.	6 men	42.6	42.2	41.7*	40.4	41.7
	3 men	39.8	43.3	40.7	41.6*	42.5
Total N in urine—grams per 24 hrs.	6 men	10.8	11.3	18.2*	10.4	12.1
	3 men	10.9	11.7	11.8	18.4*	11.2
Creatinine in urine—grams per 24 hrs.	6 men	1.57	1.59	1.65*	1.54	1.49
	3 men	1.54	1.73	1.68	1.62*	1.58
Creatinine coefficient	6 men	9.3	9.3	9.6*	8.9	8.7
	3 men	8.7	9.4	9.1	8.8*	8.7

* Values recorded during the period of gelatin feeding.

of the men in grade walking on the treadmill (table 1). The men were no more efficient during the gelatin periods than at other times and there was

very little training effect. It should be recalled that the training program was in running and did not increase the amount of walking done by the men. There were no changes of R.Q. or lung ventilation in the walk which could be related to gelatin or training. Blood lactic acid in the walk declined slightly with training.

Other metabolic processes. Table 2 shows the changes in basal metabolic rate, total nitrogen and creatinine metabolism. The basal metabolism was not affected by training or by gelatin. The subjects all took their meals at the same place and the daily diet was rather low in protein. While they were taking gelatin they showed marked increases in the total nitrogen excreted in the urine per day. The restricted protein content of their diet should have given a favorable basis for demonstrating favorable influences of additional protein.

The daily excretion of creatinine increased slightly in the first part of the training period and then declined again in the latter part. There were no significant differences during the gelatin periods. The early increase may have been associated with the building up of muscle tissue because the men made moderate gains in weight during this period. Their weight showed no consistent change during the latter half of the training period which indicates a steady state in so far as muscle growth is concerned. We have no explanation for the decline of creatinine excretion at the end.

SUMMARY

1. Nine non-athletic men were trained for running during a period of 26 weeks. Six of them took 60 grams of gelatin a day from the 9th through the 15th week of training and 3 took the same amount of gelatin from the 15th through the 21st week.

2. Changes associated with training were: consistent improvement in timed races on the track; an average increase of 16 per cent in the maximal O_2 consumption during exhausting work; an increase in the lactic acid mechanism for contracting an O_2 debt; an improvement of 8 per cent in efficiency in running; and a slight increase in the excretion of creatinine during the first part of the training period.

3. The training had no effect on the basal metabolism and caused only a slight rise of efficiency in grade walking.

4. None of the above functions were affected by gelatin. Neither the "alactacid oxygen debt" nor the lactic acid mechanism was affected by gelatin.

Acknowledgment. We are indebted to Dr. D. B. Dill of the Harvard University Fatigue Laboratory for coöperation in planning this study, and to the Research Committee of the Edible Gelatin Manufacturers of America

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THE EFFECTS OF CARBON MONOXIDE ANOXEMIA ON THE FLOW AND COMPOSITION OF CERVICAL LYMPH[1]

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This report consists of observations on the effects of anoxemia caused by the inhalation of carbon monoxide, and is a continuation of a previous report concerning anoxemia due to the inhalation of air deficient in oxygen and its effect on the production of lymph (Maurer, 1940).

The work of Campbell (1929) on the pathological effects of prolonged exposure to carbon monoxide and to very low oxygen tensions suggests that these agents may affect the production of capillary filtrate and subsequently of lymph. In his book, *Carbon monoxide asphyxia*, Drinker (1938, p. 147) quotes the work of Mayers (1930), who showed that garage workers who are more or less constantly exposed to low concentrations of carbon monoxide suffered from headache, tremors, increased reflexes and emotional instability. Drinker states that all of these effects were probably expressions of slight degrees of edema in the central nervous system. He also states (p. 156) that about one-third of all carbon monoxide poisoning cases show edema of the lungs, which is probably due to the effects of anoxemia on the lung capillaries. He believes, too, (p. 123) that the cerebral edemas resulting from carbon monoxide poisoning are due to the effects on the cerebral blood vessels of oxygen lack, which goes hand in hand with carbon monoxide poisoning. Yant, Chornyak, Schrenk, Patty and Sayers (1934) have also demonstrated that exposure to carbon monoxide caused marked cerebral edema in dogs.

The present paper deals with the result of nine experiments (eight dogs and one cat) during which the animals were first exposed to 0.5 per cent carbon monoxide in air and then to 100 per cent oxygen.

EXPERIMENTAL TECHNIQUE. All of the experiments were performed on healthy young adult animals under nembutal anesthesia (40 mgm. per kgm. intravenously). Cervical lymph was collected continuously by means of the "nodding dog" technique described by McCarrell (1939).

Pure CO was prepared by dropping concentrated formic acid into

[1] This investigation was aided by the Miriam Smith Rand Fund. The oxygen and compressed air used throughout this work were furnished through the courtesy of The Linde Air Products Company.

concentrated arsenic-free sulphuric acid. The gas was passed first through a strong solution of sodium hydroxide, then through distilled water, and was collected in a large spirometer over water after which it was compressed into a steel cylinder to a pressure of 250 pounds per square inch.

The gas mixture used in the experiments was prepared by venting first carbon monoxide and then compressed air into calibrated 80-litre spirometers, in such quantities that the resulting mixture contained 0.5 per cent CO. This mixture was delivered to the animal by means of a respiration pump at the rate of 14 inspirations per minute.

Arterial blood pressure was recorded by the usual mercury manometer. Blood samples were collected from the femoral artery and were analyzed in the Van Slyke gas analyzer for O_2, CO_2 and CO, the latter being absorbed by Winkler's cuprous chloride solution in a Hempel pipette. The details of the carbon monoxide analysis are given by Peters and Van Slyke (1932, pp. 330 336). Other experimental details were the same as those of the low oxygen and high carbon dioxide experiments previously described (Maurer, 1940).

RESULTS. *Effects on lymph flow.* It was found that the flow of cervical lymph increased during exposure to the 0.5 per cent CO-air mixture. Figure 1 illustrates the details of a typical experiment and will be referred to from time to time throughout this paper.

Lymph flow was recorded in eight dogs and one cat during exposure to the carbon monoxide mixture. In each experiment the flow of lymph showed a marked increase, the average being 2.42 times the control flow, and the range being from 1.43 to 7.5 times the control flow. The extent of the increases in the individual experiments is shown in the last column of table 1. On comparing these increases with those observed during exposure to low oxygen (table 3), one immediately notes the close agreement in the changes of lymph flow resulting from these two different methods of producing anoxemia.

The length of exposure to the 0.5 per cent CO-air mixture necessary to bring about increase in cervical lymph flow varied somewhat from animal to animal. In table 1 are recorded the lengths of exposure required by each animal to produce the changes in lymph flow which were observed. The first column of this table shows the time at which the flow of lymph began to increase, indicating therefore the point at which capillary permeability began to increase. The second column shows the time at which the increase in lymph flow became greatest, as, for example, the midpoint during carbon monoxide exposure in figure 1. The flow curve at this point turns sharply upward, marking the beginning of the period during which capillary permeability was at its maximum. The third column shows simply the time at which the flow of lymph had reached its highest point.

In the experiment illustrated in figure 1, it is seen that the attainment of maximum lymph flow was almost simultaneous with the collapse of

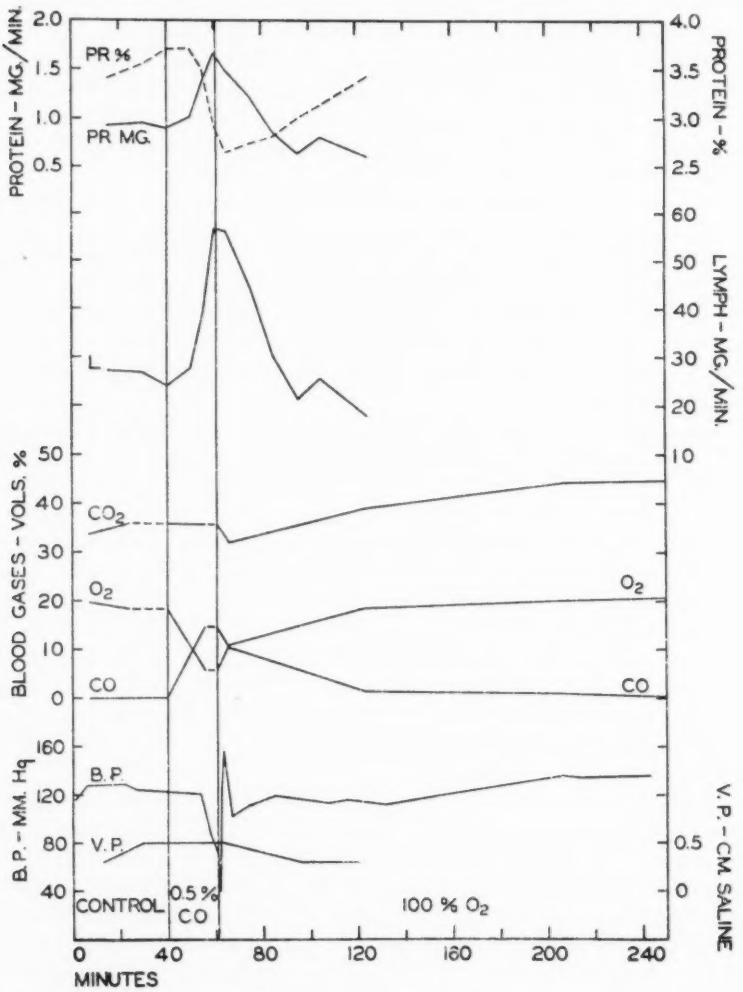

Fig. 1. Cervical lymph flow, percentage of lymph protein, output of lymph protein in milligrams per minute, blood gases and arterial and venous blood pressures of a dog exposed successively to room air (control), 0.5 per cent CO, and 100 per cent O_2.

the circulation. Such was the case in five of the experiments. In four other experiments, in which it was possible to continue carbon monoxide

exposure beyond the point at which maximum lymph flow was attained, it was observed that the flow was not maintained at the peak level even though carbon monoxide continued to be administered, but reversed itself sharply and had considerably diminished before the circulation showed signs of failure. In this respect also the effect of carbon monoxide parallels the effect of exposure to low oxygen (Maurer, 1940). It has already been pointed out in the low oxygen paper that this reversal of lymph flow was undoubtedly caused by the increase in colloid osmotic pressure of the blood serum resulting from the great loss of fluid expressed as increased lymph production.

Oxygen saturation in relation to lymph flow. Table 2 shows the oxygen and carbon monoxide saturation of the arterial blood of the nine animals

TABLE 1

Exposure to 0.5 per cent carbon monoxide necessary to change cervical lymph flow and magnitude of the maximum increase

DOG NO.	INCREASED FLOW	STEEPEST FLOW	MAXIMUM FLOW	
	Exposure	Exposure	Exposure	Increase over normal
	minutes	*minutes*	*minutes*	*times*
1	10	20	35	1.8
2	20	20	35	1.6
3	5	15	25	1.9
4	30	40	45	1.6
5	10	10	30	7.5
6	20	20	40	1.6
7*	5	10	20	2.1
8	10	15	20	2.3
9 (cat)	10	10	20	1.4
Average	13	18	30	2.4

* This is the experiment shown in figure 1.

on which the flow of lymph was recorded. It will be noted in this table that the sum of the oxygen and carbon monoxide saturations at any given state of lymph flow is in most cases greater than 100 per cent. This is undoubtedly due to the fact that during CO poisoning the spleen contracts and expels a considerable quantity of red cells into the circulating blood in an effort to reduce the proportion of CO hemoglobin in the general circulation (de Boer and Carroll, 1924; Campbell, 1932; Naismith and Graham, 1906).

It is interesting to note the values for the average oxygen saturation in this group of experiments. The close similarity of these figures to those obtained during exposure to low oxygen (table 3) is especially striking. This remarkable parallelism of oxygen saturation in the two series of experi-

ments brings out quite clearly that the effect of carbon monoxide, at least in regard to the flow of lymph and therefore the permeability of the capillaries, is in all probability not due to the presence of carbon monoxide in

TABLE 2

*Relation of cervical lymph flow to oxygen and carbon monoxide saturation of arterial blood**

DOG NO.	INCREASED FLOW		STEEPEST FLOW		MAXIMUM FLOW	
	O_2 saturation	CO saturation	O_2 saturation	CO saturation	O_2 saturation	CO saturation
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	75.7	30.0	48.6	59.5	16.2	97.3
2	48.8	58.5	48.8	58.5	14.6	92.6
3	81.0	19.0	52.4	52.4	23.8	80.9
4	40.0	70.0	20.0	95.0	15.0	100.0
5	64.1	33.3	64.1	33.3	33.3	71.8
6	42.4	66.7	42.4	66.7	18.1	97.0
7†	73.7	26.3	52.6	47.3	21.0	78.9
8	55.3	51.1	30.0	76.6	17.0	93.6
9 (cat)	64.3	28.6	64.3	28.6	28.6	71.4
Average..	60.6	42.6	47.0	57.5	20.8	87.1

* Carbon monoxide saturation is calculated on the basis of the normal blood oxygen content of each animal.

† This is the experiment shown in figure 1.

TABLE 3

Relation of cervical lymph flow to blood oxygen saturation and altitude (Maurer, 1940)

EXPERIMENT	INCREASED FLOW		STEEPEST FLOW		MAXIMUM FLOW	
	O_2 saturation	Altitude	O_2 saturation	Altitude	O_2 saturation	Increase over normal
	per cent	*feet*	*per cent*	*feet*	*per cent*	*times*
1	70.7	18,500	46.3	>20,000	14.6	3.3
2	76.7	16,500	62.5	>20,000	17.0	3.2
3	82.6	14,000	75.9	17,000	69.0	3.9
4	69.8	19,000	40.7	>20,000	12.5	1.8
5	74.5	17,500	45.2	>20,000	36.0	1.6
6	75.5	17,000	44.3	>20,000	13.0	2.6
Average...	75.0	17,000	52.5	>20,000	27.0	2.7

the blood *per se*, but to the low oxygen saturation resulting directly from the high carbon monoxide content. This finding is in accord with the work of Haldane and Smith (1896), who stated that the effects of CO poisoning were due to oxygen deprivation and not to the CO itself. They

believed that carbon monoxide acted only as an inert gas, such as nitrogen, and aside from rendering the hemoglobin incapable of carrying oxygen, it had no other effect. Haggard (1921), in studying the effects of CO asphyxia on the heart, suggested likewise that this gas exerted no toxic action but that the effects observed were due to anoxemia alone.

Carbon dioxide content of the blood. In the experiment of figure 1, the CO_2 content of the blood remained practically constant during the period of exposure to carbon monoxide, but fell approximately 4 volumes per cent during the five minutes directly following. In each of the other eight experiments of this series, the CO_2 content of the blood decreased considerably as the carbon monoxide content increased. In fact, the decrease of the CO_2 in five instances was as great as the decrease of the oxygen content. In the whole series of experiments, the extent of the CO_2 decreases ranged from 3 volumes per cent to 24 volumes per cent.

Haggard and Henderson (1921) have shown that as carbon monoxide poisoning progressed the CO_2 content of arterial blood decreased along with a marked increase in the respiratory volume. They pointed out at the same time that the CO_2 combining power of the blood also diminished somewhat. Drinker (1938, p. 27), in discussing this report, remarked that the fall of CO_2 was due to the increased respiratory volume and the consequent blowing-off of CO_2. In the present series of experiments, however, the animals were rendered incapable of voluntary respiration by the administration of curare in order that lymph flow would not be affected by the excessive muscular movements which accompany asphyxia. At the same time the respiratory rate and volume remained constant since respiration was controlled by a mechanical respiratory pump. Even so, six of these animals showed marked decreases (6 to 24 volumes per cent) of their arterial CO_2 content, the other three showing smaller decreases (3 to 4 volumes per cent). This observation suggests, in agreement with the work of Kamei (1931), that though hyperventilation may be observed in many instances, it may not be "solely responsible for the decrease of the carbon dioxide content of the arterial blood in an animal intoxicated by means of carbon monoxide." It is entirely possible that the CO_2 as well as the O_2 content of the blood is decreased as a result of the formation of CO hemoglobin and the consequent loss of CO_2 combining power, or, as Kamei suggests, the CO_2 decrease may be the result of acidosis which may accompany CO poisoning.

Lymph protein. Total protein was determined by means of the Zeiss dipping refractometer calibrated against known samples of dog serum and lymph. The data are in agreement with findings reported many times from this laboratory, and with observations on the effects of low oxgen and high carbon dioxide (Maurer, 1940), namely, that with increased lymph flow the percentage of lymph protein decreases while the protein

output in milligrams per minute increases, and that these values return to normal as flow returns to normal. The experiment of figure 1 is typical of the results obtained. Though serum protein was not followed in this particular experiment, other experiments of this series showed a decreasing percentage of serum protein accompanying the increased output of lymph protein which always occurred during the periods of increased lymph flow.

Arterial and venous blood pressures. The arterial pressure curve of the experiment in figure 1 is quite typical of the eight dog experiments performed. In one instance, however, the rapid fall of pressure was preceded by a slow rise of 10 mm. of mercury during the first ten minutes of exposure to carbon monoxide, after which the pressure fell sharply as in figure 1.

Observations of the arterial pressure of five cats (including the cat of the present series) during exposure to 0.5 per cent CO revealed pressure curves like that of figure 1 in only two cases. The other three cats showed much the same sort of curves that are seen on exposure to low oxygen, namely, a sharp initial increase of 20 to 30 mm. of mercury, lasting for only a brief interval and followed by an immediate sharp fall.

Venous pressure was recorded in the external jugular vein during four of the dog experiments. The venous pressure in figure 1 shows no change during the period of carbon monoxide exposure. During the other three experiments there were increases in venous pressure ranging from 0.3 to 0.8 cm. of saline, the greater part of the increase occurring toward the end of the period of exposure. It seems hardly possible that the changes observed in arterial or venous pressures could be responsible in any way for increased lymph flow (Maurer, 1940).

Recovery with 100 per cent oxygen. In each of the experiments inhalation of CO was continued until the arterial blood pressure had reached a dangerously low level, at which time each animal was exposed to 100 per cent O_2. Three of the animals (all dogs) did not respond to this treatment, the exposure to CO having progressed to the point of complete collapse of the circulation. The other animals, however, responded to this treatment more or less adequately.

The seven animals which survived exposure to CO and which were treated with 100 per cent O_2 may be divided into two groups. In the first group, consisting of three dogs and the cat, oxygen treatment was begun when lymph flow had reached its peak. The reason for this coincidence lies in the fact that it was at this particular point that circulatory collapse was imminent, and that in order to continue the experiment oxygen treatment had to be initiated. The experiment of figure 1 is typical of this group in this respect. In each of the four experiments of group 1, lymph flow was immediately reversed and fell throughout the remainder of each experiment until the rate of flow had reached the normal value or slightly

below the normal. The length of time from the beginning of oxygen treatment until the flow had reached the control level ranged from 17 to 30 minutes. In figure 1 the flow decreased sharply, reaching the control level within 30 minutes, but continued, with the exception of one slight upward fluctuation, to fall until it was considerably below the control level after 65 minutes of oxygen inhalation.

In the second group, consisting of three dogs, the flow of lymph had reached its maximum point and had fallen approximately half the distance to the control level before there was any danger of circulatory collapse. Oxygen treatment did not begin in these animals, therefore, until 6 to 25 minutes after maximum flow had been attained. In each animal lymph flow continued to decrease during the inhalation of oxygen until it had fallen to, or slightly below, the control level.

With respect to lymph flow alone, it would be difficult to state whether or not the inhalation of 100 per cent O_2 had any beneficial effects in any of the animals of either group. In the animals of group 2, lymph flow had already decreased considerably before oxygen inhalation had begun, probably as the natural result of the increased osmotic pressure which must necessarily have followed the great loss of fluid from the circulating blood during the period of increased lymph flow. However, it is certainly true that not a single one of the seven treated animals, especially those of group 1, would have survived had oxygen therapy not been instituted when the arterial blood pressure had become dangerously low. Whether or not, then, the return of lymph flow to normal depended upon any beneficial effect exerted by the oxygen, it can be stated that undoubtedly it was exceedingly beneficial in prolonging the lives of these animals.

Certainly without the aid of oxygen, circulation would have been restored in none of these animals. Depending on the depth of the CO asphyxia, arterial blood pressure increased more or less rapidly. In most instances, of which figure 1 is again a typical example, arterial pressure returned to or nearly to normal within the first five minutes of oxygen inhalation. In one experiment blood pressure remained at a very low level for nearly an hour with only a very slight increase during that time, and then rose to normal only after a trace of ephedrine was given intravenously. Following this injection the pressure remained normal until the experiment was terminated an hour and a half later.

In every case oxygen saturation began to increase immediately, and had become normal in from 40 to 95 minutes. In most instances the oxygen content of the arterial blood was somewhat higher than the content of the normal control blood. This increase of oxygen saturation over normal has already been explained as the result of the expulsion of red cells from the spleen into the circulating blood during the course of carbon monoxide poisoning.

The CO content of the blood began to decrease immediately with the beginning of oxygen inhalation. This decrease was more rapid in some animals than in others. After from 40 to 95 minutes most of the CO had been given off, there remaining only 1 to 2 volumes per cent. These last 1 or 2 volumes of CO were given off exceedingly slowly, for even though the inhalation of oxygen continued another two hours, there still remained traces of CO in the blood.

As a general rule, the CO_2 content of the blood was restored to normal somewhat more slowly than was the O_2 content, though with one or two exceptions it had been completely restored before the experiments were terminated.

Discussion. There can be little doubt that poisoning with carbon monoxide causes capillary damage and that this damage is expressed in increased lymph production.

At the same time, after comparing the effects of carbon monoxide and low oxygen anoxemias on lymph production, it seems entirely reasonable to believe that carbon monoxide brings about such changes only by diminishing the oxygen-carrying capacity of hemoglobin. It is not too remarkable, therefore, that these two different types of anoxemia should show increased lymph production at so nearly the same levels of blood oxygen saturation.

Even though treatment with 100 per cent oxygen is accompanied by the return of lymph production to normal levels, it cannot truly be said that the oxygen was responsible, and it cannot be denied that lymph flow would very likely have returned to normal due to the influence of other factors. Certainly, oxygen was beneficial in restoring arterial blood pressure, and at the same time was responsible for the return to normal of the blood gases.

The author wishes to take this opportunity to thank Dr. Cecil K. Drinker for suggesting this problem and for his helpful advice and criticism throughout the work; and also to thank Miss Anne C. Messer for technical assistance with gas analysis.

SUMMARY

Experiments are reported in which exposure of dogs and cats to 0.5 per cent CO resulted without exception in increased production of cervical lymph. The average increase in flow was 2.42 times the control flow, the range being from 1.43 to 7.5 times the control flow.

The increase in lymph production began when the average oxygen saturation was 61 per cent, which compares closely with results obtained during exposure to air deficient in oxygen, and would confirm the belief that CO is of itself non-toxic, acting only through its ability to reduce oxygen-carrying capacity.

Treatment with 100 per cent O_2 resulted in restoration of arterial blood pressure and blood gases to normal levels, and was accompanied in part by the return to normal of lymph production.

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THE EFFECTS OF ANOXEMIA DUE TO CARBON MONOXIDE AND LOW OXYGEN ON CEREBROSPINAL FLUID PRESSURE[1]

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A preceding paper, concerned with the effects of carbon monoxide anoxemia on the flow and composition of cervical lymph, was followed by observations upon the effects of this agent, as well as of low oxygen, on the cerebrospinal fluid pressure of cats. Other workers have studied cerebrospinal fluid pressure under similar conditions, but no data have been published which correlate the effects observed with either the carbon monoxide or oxygen saturation of the blood.

Forbes, Cobb and Fremont-Smith (1924) have reported that the cerebrospinal fluid pressure of cats and dogs increases markedly during exposure to carbon monoxide. They did not, however, report any data concerning the degree of CO or O_2 saturation of the blood at any time during the period of increased pressure. They did report the results of an experiment on a man who inhaled 0.2 per cent CO for 35 minutes. The subject became dizzy, sick and weak, with a sense of fulness in the head and a dimming of vision, but with no real headache until 10 minutes after the gassing had been stopped. At the end of the 35 minutes' gassing, a tannic acid test for CO hemoglobin showed 40 per cent CO saturation. They concluded from this experiment that the headache resulted from increased pressure in the cerebrospinal canal.

Other investigators have reported that the administration of gaseous mixtures low in oxygen also brings about increases in the pressure of the cerebrospinal fluid in animals (Hill, 1896; Nicholson, 1932; Yesinick and Gellhorn, 1939). Michelsen and Thompson (1938) have observed clinical manifestations of increased intracranial pressure following exposure to oxygen tensions corresponding to an altitude varying between 15,500 and 17,000 feet. Concerning the blood gases, they make the statement that "the oxygen saturation of the blood of the subjects during the stay in the chamber varied in different persons between approximately 50 and 70

[1] This investigation was aided by the Miriam Smith Rand Fund. The oxygen and compressed air used throughout this work were furnished through the courtesy of The Linde Air Products Company.

per cent, whereas the atmospheric O_2 tension was reduced to the same degree for all individuals."

It is the purpose of the present report to attempt to show the relation between the degree of CO and O_2 saturation of the blood and cerebrospinal fluid pressure.

EXPERIMENTAL TECHNIQUE. The experiments were performed on seven healthy young adult cats under nembutal anesthesia (40 mgm. per kgm. intraperitoneally). The source of carbon monoxide was the same as that described in the previous paper (Maurer, 1941). The gas was diluted to 0.5 per cent with air, and was delivered from 80-liter spirometers to the animal by means of a respiration pump at the rate of 14 inspirations per minute.

One femoral vein was cannulated to facilitate the administration of various experimental agents. The femoral artery of the opposite leg was cannulated for recording arterial pressure and for collection of blood samples. The O_2, CO_2, and CO content of these samples was determined by means of the Van Slyke gas analyzer. Complete details for the analysis of carbon monoxide are given by Peters and Van Slyke (1932, pp. 330–336). The animals were also curarized (0.4 to 0.6 cc. of a 1 per cent solution by vein, depending upon body weight) to prevent any random effects on cerebrospinal fluid pressure by excessive muscular movements of the thorax or diaphragm.

With the animal lying on its back, the hind legs were strapped to a board. The upper body and head were then turned onto one side so that the back of the head and neck were completely exposed to the operator. A large gauge needle, fitted with a stilette, was then pushed through the occipito-atlantoid ligament into the cisterna magna. After removing the stilette, the needle was connected by a flexible rubber tube to a vertical glass tube of 3.5 mm. bore, which was fastened to a meter stick. This system was then filled with physiological saline solution to a height corresponding to the average cerebrospinal fluid pressure (6 to 8 cm.).

RESULTS. *Carbon monoxide experiments.* Carbon monoxide was used in five experiments. Though the cerebrospinal fluid pressure rose in each case, the experiments seem to fall into two groups.

In the first group there are four experiments, in each of which there was an almost immediate increase in pressure when the 0.5 per cent CO was administered. Figure 1 illustrates this response. Figure 1 also illustrates an observation made in two of these experiments, namely, that there was a rapid increase of pressure followed by a short interval during which the pressure fell slightly and following which it again increased sharply. The time at which these secondary increases began was 11 and 13 minutes, respectively, after gassing had begun (fig. 1, 13 min.). In the third of this first group of experiments, the pressure began to increase almost

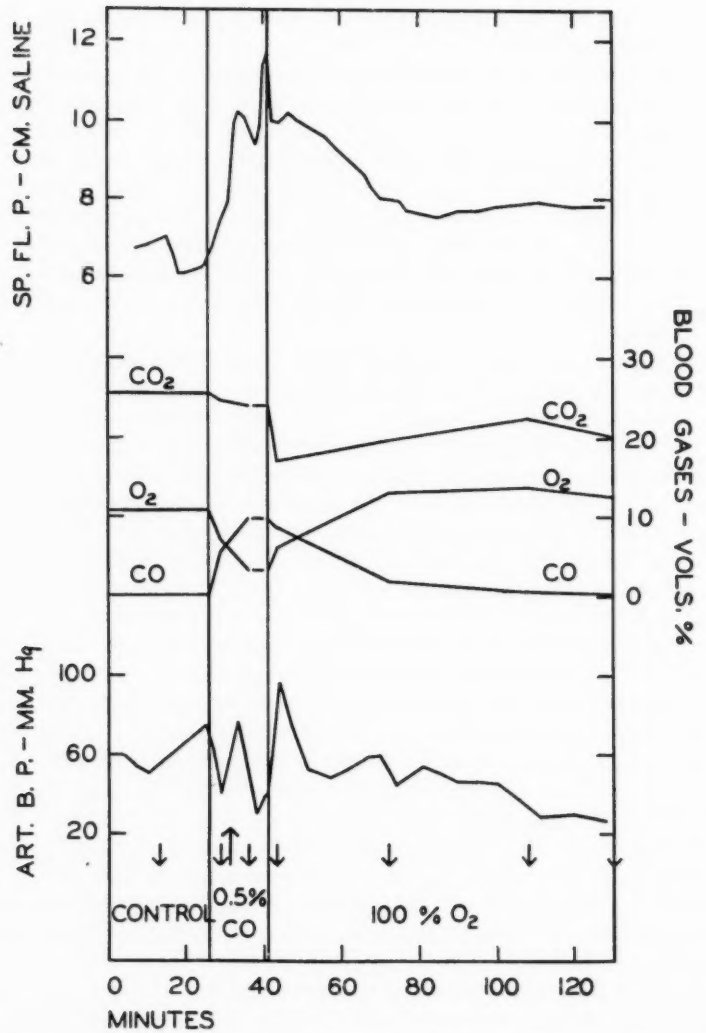

Fig. 1. Cerebrospinal fluid pressure, blood gases, and arterial blood pressure of a cat exposed successively to room air (control), 0.5 per cent CO, and 100 per cent O_2. The arrows pointing downward indicate the times at which blood samples were taken. The arrow pointing upward indicates the time at which a transfusion of 25 cc. of cat blood was given.

immediately but rose only 0.4 cm. of saline in 10 minutes. This slight rise was followed by an 8 minute interval during which there was no change.

Then abruptly the pressure increased sharply. In the fourth experiment, the pressure began to increase almost immediately but continued to rise steadily without a break.

In the single experiment of the second group, the pressure began to rise only after the CO had been on for 5 minutes, and it then rose only 0.5 cm. of saline during the next 13 minutes, at which time the animal died.

In each of these experiments the pressure increased as the gassing continued, until it had reached a maximum or until the circulation showed signs of failure, at which time the gassing was discontinued and 100 per cent oxygen was administered, as illustrated in figure 1. Table 1 shows for each experiment the extent of the pressure increase, the length of exposure to CO, and the degree of O_2 and CO saturation of the blood at the

TABLE 1

Relation of oxygen and carbon monoxide saturation to cerebrospinal fluid pressure

EXPERIMENT (CATS)	BEGINNING OF INCREASED PRESSURE			SECONDARY INCREASE OF PRESSURE			MAXIMUM PRESSURE			
	Length of exposure	Arterial saturation		Length of exposure	Arterial saturation		Length of exposure	Arterial saturation		Increase over normal
		O_2	CO		O_2	CO		O_2	CO	
	minutes	*per cent*	*per cent*	*minutes*	*per cent*	*per cent*	*minutes*	*per cent*	*per cent*	*times*
1	Immediate	100	Negligible	18	29	100	23	21	107	2.58
2*	Immediate	100	Negligible	13	32	94	16	30	100	1.80
3	Immediate	100	Negligible	11	63	48	16	52	65	1.70
4	Immediate	100	Negligible				18	36	60	1.56
5	5	53	45				18			1.05
Average..				14	41	81	18	35	83	1.74

* This is the experiment of figure 1.

time of the secondary pressure increase and at the time of maximum pressure.

It will be noted in this table, just as in table 2 of the preceding paper (Maurer, 1941), that the sum of the O_2 and CO saturations at any given time is in most cases greater than 100 per cent, which is undoubtedly due to the expulsion from the spleen of quantities of red cells into the circulating blood in an effort to reduce the proportion of CO hemoglobin in the general circulation (de Boer and Carroll, 1924; Campbell, 1932; Naismith and Graham, 1906).

Recovery with 100 per cent oxygen. In each of these experiments maximum cerebrospinal fluid pressure was not attained until the arterial blood pressure had become dangerously low. At this point the administration of CO was stopped and treatment with 100 per cent O_2 was begun. Three

of the five animals responded almost immediately with greatly increased arterial blood pressure. The animal of experiment 3 did not respond to this treatment, dying 3 minutes after it was begun; and the animal of experiment 5 was dead before oxygen could be administered. In the three experiments in which the animals recovered, the cerebrospinal fluid pressure fell sharply at first and then decreased more and more gradually until it had reached a level somewhat higher than the original pressure. Figure 1 illustrates the decrease of pressure during 87 minutes of oxygen administration in one of these experiments, while table 2 shows the results of oxygen administration for all of the experiments.

It will be noted that in none of these experiments did the pressure return to normal during the period of oxygen treatment, though undoubtedly it would have done so had the experiments been greatly prolonged. This

TABLE 2

Effect of 100 per cent oxygen on increased cerebrospinal fluid pressure

EXPERIMENT (CATS)	CEREBROSPINAL FLUID PRESSURE		100 PER CENT O_2 TREATMENT	
	Normal	Maximum	Length of exposure	Final cerebrospinal fluid pressure
	cm. saline	*cm. saline*	*minutes*	*cm. saline*
1	3.6	9.3	47	6.8
2*	6.5	11.7	87	7.8
3	9.1	15.5	3	12.5†
4	5.5	8.6	17	6.0
5	9.2	9.7		
Average.....	6.8	11.0		

* This is the experiment of figure 1.

† At death.

rather slow decrease of the cerebrospinal fluid pressure is probably explained by the fact that absorption from the cerebrospinal canal goes on normally at a slow rate. Forbes, Cobb and Fremont-Smith (1924) demonstrated that recovery from carbon monoxide poisoning could be greatly increased by the injection of hypertonic saline solution intravenously. The present experiments show that treatment with oxygen alone is not nearly as effective as hypertonic saline alone or with oxygen, particularly with regard to cerebral edema and the resulting increased intracranial pressure.

Low oxygen experiments. Cerebrospinal fluid pressure was recorded in two experiments in which the animals were exposed to 8 per cent and 6 per cent oxygen, respectively. In both experiments the pressure rose immediately. The first animal was exposed to 8 per cent oxygen for 52 minutes. The cerebrospinal fluid pressure increased immediately along with the characteristic short increase in arterial pressure which accompanies expo-

sure to low oxygen. This pressure continued to increase, however, even in the face of the rapidly diminishing blood pressure, and remained at its new height until 15 minutes before death, when it fell off sharply with the terminal collapse of the circulation.

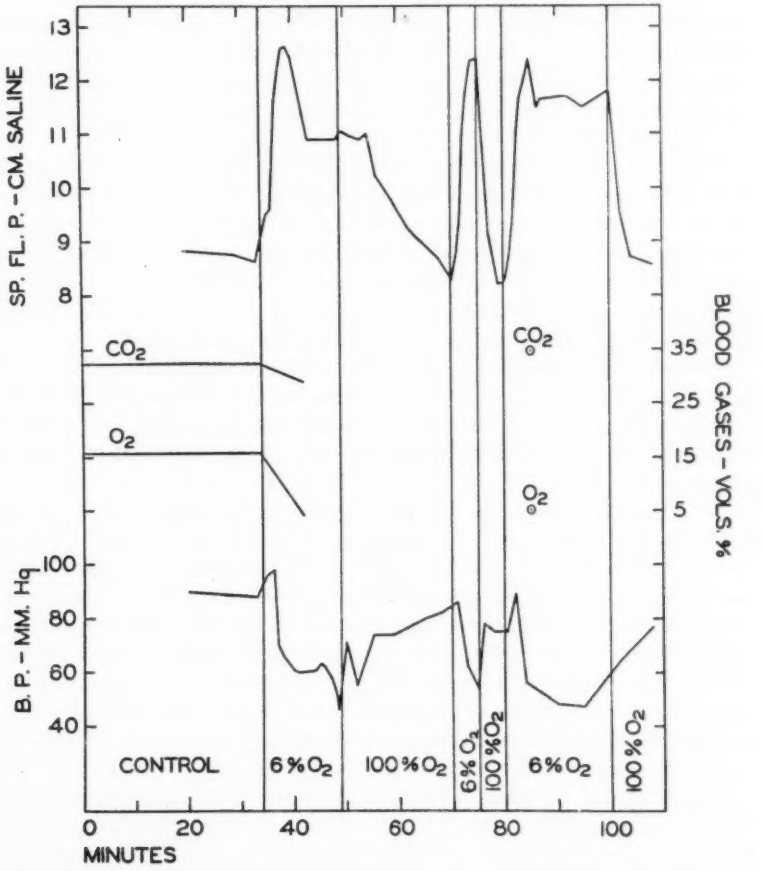

Fig. 2. Cerebrospinal fluid pressure, blood gases, and arterial blood pressure of a cat exposed alternately to 6 per cent O_2 and 100 per cent O_2.

The results of exposure to 6 per cent oxygen in the second experiment are illustrated in figure 2. Here again is seen the sharp increase in cerebrospinal fluid pressure which continues even after the arterial blood pressure is greatly diminished. In this experiment the animal was exposed alternately to 6 per cent and 100 per cent oxygen three times. Each exposure

to low oxygen was followed by an immediate increase in cerebrospinal fluid pressure amounting to 4 cm. of saline. During the two longer exposures the pressure remained considerably elevated until the administration of 100 per cent oxygen was begun. Treatment with high oxygen in this particular animal resulted in a rapid return of the cerebrospinal fluid pressure to its normal level. This rapid return of pressure following administration of low oxygen is characteristic, and differs from the slower return following exposure to carbon monoxide because in the latter case a certain degree of anoxemia persists until the greater part of the CO has been eliminated.

Discussion. Forbes, Cobb and Fremont-Smith (1924) concluded from their experiments on animals that the sudden sharp increases in cerebrospinal fluid pressure were due to cerebral congestion which accompanied the initial increase in arterial blood pressure. They demonstrated this point by observation of dilatation of the retinal blood vessels. They also showed that the prolonged effects of carbon monoxide on intracranial pressure were due to increased brain volume, which could be readily diminished by the intravenous injection of hypertonic saline. This would lead to the conclusion that prolonged elevation of intracranial pressure was due to the accumulation of fluid in the brain tissue. Indeed, they showed this to be the case by desiccating the brains of normal and of poisoned animals.

In the present work the retinal vessels were not observed, but it was noted in these experiments and in those of the preceding paper (Maurer, 1941), that exposure to carbon monoxide was in a number of cases accompanied by short initial increase in arterial pressure similar to the increase which accompanies exposure to low oxygen. Since it is a fact that changes in the circulation of a local area can occur even though similar changes are not seen at the same time in the general circulation, it is entirely possible that exposure to carbon monoxide would be accompanied by increased cerebral blood pressure even though an increased pressure was not recorded in the femoral artery.

It was shown in table 1 that cerebrospinal fluid pressure began to increase before there could have been any effective diminution of the arterial oxygen saturation. It was also shown that in three experiments there was a secondary increase of pressure which occurred when the average oxygen saturation had reached 41 per cent. This figure compares very favorably with the figures for oxygen saturation when cervical lymph flow shows its sharpest increase during exposure to low oxygen (Maurer, 1940) and during exposure to carbon monoxide (Maurer, 1941).

Table 3 shows a résumé of the degrees of oxygen saturation which have been observed during changes in cervical lymph flow and cerebrospinal fluid pressure. In agreement with Forbes, Cobb and Fremont-Smith

(1924), these data lead to the conclusion that the initial increase in cerebrospinal fluid pressure is in all probability due to cerebral congestion resulting from increased arterial pressure. The data also show that without doubt the secondary increase and the prolonged elevation of intracranial pressure during and following exposure to carbon monoxide or low oxygen are due to the accumulation of fluid in the brain tissue. It has already been shown that decreased oxygen saturation results in increased capillary permeability (Maurer, 1940, 1941) and the resultant loss of considerable quantities of fluid from the circulating blood. It is considered significant, therefore, that the degree of oxygen saturation during the period of sharpest increase in lymph flow is so nearly similar to the oxygen saturation at the time of the secondary increase in cerebrospinal fluid pressure. It is also significant

TABLE 3

Relation of oxygen saturation to lymph flow and cerebrospinal fluid pressure

OBSERVATION	LYMPH EXPERIMENTS		CEREBROSPINAL FLUID PRESSURE EXPERIMENTS	
	With low O_2	With CO	With CO	With low O_2
	per cent oxygen saturation			
Beginning of cerebrospinal fluid pressure increase			100	100
Beginning of increased cervical lymph flow	75	61		
Sharpest lymph flow	53	47		
Secondary cerebrospinal fluid pressure increase			41	
Maximum lymph flow	27	21		
Maximum cerebrospinal fluid pressure			35	28 44 Av. 36

that the degrees of oxygen saturation during the maxima for lymph flow and cerebrospinal fluid pressure are within the same range.

The author wishes to take this opportunity to thank Dr. Cecil K. Drinker for his helpful suggestions and criticisms throughout this work; and to thank Miss Anne C. Messer for technical assistance in gas analysis.

SUMMARY

Experiments are reported in which exposure of cats to 0.5 per cent CO and to 6.0 and 8.0 per cent O_2 resulted without exception in increased cerebrospinal fluid pressure. The average increase during CO exposure was 1.74 times the normal. The increases during 6.0 and 8.0 per cent O_2 were 1.5 and 1.1 times the normal, respectively.

The immediate increases in cerebrospinal fluid pressure are believed to be due to increased cerebral blood pressure, since the change occurs before the blood O_2 saturation is effectively lowered.

The secondary increase and the prolonged elevation of cerebrospinal fluid pressure are believed to be due to accumulation of fluid from the cerebral capillaries, whose permeability is increased when blood O_2 saturation is effectively lowered.

The O_2 saturation during the secondary pressure increase averages 41 per cent, and at the time of maximum pressure averages 35 per cent.

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